ANNUAL REPORT

Division of Intramural Research Programs National Institute of Mental Health

October 1, 1983 - September 30, 1984

VOLUME I SUMMARY STATEMENTS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

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ANNUAL REPORT
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DIRECTOR, DIVISION OF INTRAMURAL RESEARCH PROGRAMS
October 1 ,1983 - September 30, 1984
Frederick K. Goodwin, MD

This, my third annual report as Scientific Director, covers a period of relative stability for the Intramural Program, following the earlier upheavals associated with our reorganization. On the other hand, leadership at both the ADAMHA and Institute levels was in transition during this year. The prolonged uncertainty has now ended happily with the selection in June of Dr. Donald Macdonald as ADAMHA Administrator and in September of Dr. Shervert Frazier as NIMH Director.

Soon after his arrival last Spring, Dr. Macdonald immersed himself in the Substance of Intramural research, with three lengthy scientific briefings and many informal contacts. His easy rapport with investigators and quick grasp of research issues has been impressive and we wish him well in his stewardship of this major health agency.

The appointment of Dr. Frazier, formerly Professor of Psychiatry at Harvard, and the departure of his predecessor Dr. Herbert Pardes to become Professor and Chairman of Psychiatry at Columbia, underscores the strong ties between our Institute and the academic community. As Director of Harvard's McLean Hospital, Dr. Frazier helped to build a distinguished research facility headed by Dr. Seymour Kety (now IRP Associate Director for Basic Science). Seymour will attest to his old -- and new -- boss's prowess as a research advocate. We are looking forward to welcoming Dr. Frazier to the IRP and to familiarizing him with our work.

One of Dr. Macdonald's first official functions as Administrator Designate was to address our IRP banquet honoring Dr. Julius Axelrod on June 1. Julie officially retired from Federal service this year, but is continuing his research as a guest worker in Dr. Mike Brownstein's Laboratory of Cell Biology. The banquet culminated a memorable two-day symposium on "Mechanisms of Synaptic Regulation," which brought together hundreds of Julie's associates from around the world to share findings and celebrate the remarkable career of NIMH's Nobel Laureate. The banquet brought together scientific and science policy leaders with Julie's friends and colleagues, old and new. Policy leaders in attendance included Assistant Secretary of Health Dr. Ed Brandt, Assistant Secretary for Personnel Tom McFee (who delivered a special citation from Secretary Heckler), White House Science Advisor Dr. George Keyworth, Senator Thomas Eagleton and Congressman Stenny Hoyer. The whole affair -- from program substance to travel logistics to menu planning -- was tastefully and meticulously orchestrated by Dr. Marty Zatz. Underwritten by Intramural funds and individual and corporate contributions, the Axelrod event spotlighted the Intramural Program's science and values for health leadership throughout the Government. Dr. Keyworth and his aide, Bernadine Bulkley, showed a keen interest in the Program during subsequent briefings on campus.

Following in the same tradition of excellence personified by Julie Axelrod is a distinguished group of Intramural staff members who received major awards over the past year. These include Dr. Mortimer Mishkin, who became our eighth member of the National Academy of Sciences; Dr. Edward Evarts, the SES Presidential Meritorious Executive Rank Award; Dr. Seymour Kety, the first American ever to win West Germany's Golden Kraepelin Medal, and also the first recipient of Japan's new Mihara Foundation Award; Dr. Richard Wyatt, the American College of Neuropsychopharmacology's Daniel Efron Award; Dr. Erminio Costa, Italy's Gold Medal for outstanding cultural contributions; Dr. Philip Gold, the C.P. Richter Prize in Psychoneuroendocrinology; Dr. John Morihisa, the A.E. Bennett Neuropsychiatric Research Foundation Award; Dr. Carmi Schooler, election to membership in the Sociological Research Association; Dr. Melvin Kohn, election to Fellow status in the American Association for the Advancement of Science; Dr. Giulio Cantoni, the DHHS Distinguished Service Award; Dr. Theodore Colburn, the PHS Superior Service Award; and Dr. Thomas Wehr, the PHS Meritorious Service Medal. I was indeed honored to receive the first Distinguished Alumni Award from the Department of Psychiatry at the University of North Carolina.

Deserving of special mention has been Dr. John Eberhart's remarkable recovery this past year. It has indeed been inspiring to see our former director's indomitable spirit and perseverance buoy him through a 10-month seige of a rare, serious illness here at the Clinical Center. We all celebrate his return home and look forward to his speedy return to duties in the NIH Director's office, where John's presence symbolizes the close ties between our program and the greater NIH community.

Since succeeding John as NIMH Scientific Director, I have attempted to make the most of what we have through reorganizing units, shifting personnel, and reallocating space -- all within the constraints of existing resources. The space realignments, now completed, have been carried out according to the principle of equity and couldn't have been accomplished without the cooperation of the lab/branch chiefs and the tireless efforts of Drs. Ted Colburn and Bill Potter.

Dr. Mike Brownstein's Laboratory of Cell Biology (LCB) has moved from Building 10 to Building 36, enlarging our critical mass of basic neuroscientists in that building. A new Laboratory of Molecular Biology, headed by Dr. David Neville, brings together his section with those of Drs. Howard Nash and Werner Klee. This unites a group of first class scientists working in closely related areas of molecular genetics, membrane biology and structure and function of receptors.

Leaving the Laboratory of General and Comparative Biochemistry and moving to Building 10 this year is Carl Merril's section, which joins the Clinical Psychogenetics Branch, strengthening its ability to conduct coordinated clinical and basic research.

Among major organizational changes, the Laboratory of Clinical Science, now under Dr. Dennis Murphy, has incorporated Dennis' old Neuropharmacology Branch, making the LCS again our largest lab. In recognition of her unquestioned position at the forefront of research in child psychiatry, Dr. Judy Rapoport has been elevated to chief of a new Child Psychiatry Branch, with remodeled quarters on the 6th floor of the Clinical Center.

Development of our joint NICHD/NIMH primate facility at Poolesville is progressing, with the first group of Dr. Steve Suomi's rhesus monkeys having arrived from Wisconsin last spring. Opening of a naturalistic, 5-acre outdoor habitat was marked by a brief ceremony and press briefing in early June. Construction continues on the indoor monkey facility. Dr. Thomas Insel, coordinator of the NIMH component is, among other things, raising a colony of tiny marmoset monkeys for studies at the Poolesville center. Other campus-based NIMH investigators who are taking advantage of the Poolesville resource include Drs. Jacqueline Crawley, Thomas Uhde, John Glowa, Sidney Werkman and James Hill.

Dr. Paul MacLean, the real "father" of our Poolesville facility, has joined the Laboratory of Clinical Science as a senior scientist but will remain in Poolesville as a senior advisor to our scientists and those of NICHD. Dr. Ichiji Tasaki has become a senior scientist in Dr. Evart's Laboratory of Neurophysiology.

Unfortunately, even efficient management of resources cannot entirely make up for the erosion in financial and personnel support the Intramural Program has sufffered over the past decade and a half. In 1968, our full-time permanent ceiling was cut by 100 and this year we lost 29 positions. Yet during this same period, growth in the brain sciences and in techniques for translating this new knowledge into clinical insights has been unprecedented. The Intramural Program's true discretionary monies, the "other objects" funds that support research, have chronically been only 60% of the average for NIH Institutes of comparable size. The formula determining NIMH's contribution to the NIH Management Fund has for years inadvertently resulted in the NIMH bearing about $2 million dollars more than its share of the NIH's "overhead" costs, largely for the support of the Clinical Center. Fortunately, this formula is now under revision and is expected to reflect more realistic cost estimates in future years. An ostensible $3.7 million budget increase in FY 1983, upon closer examination, turns out to be spurious; a bookkeeping change simply shifted the budget for the overhead costs of our program at Saint Elizabeths Hospital from the NIMH Director's Office to the Intramural Program.

Of course, parallel discrepancies between NIMH and the NIH Institutes are similarly conspicuous in extramural grant funded research. Mental health research as a whole has yet to recover from the funding doldrums of the 1970's. We will need an infusion of new resources if we are to seize the opportunities arising in neuroscience and keep pace with the increasing costs of research technologies.

Positron Emission Tomography represents a prime example. Our new $1.7 million PET scanner is scheduled to be completed this Spring. Drs. Richard Coppola and Robert Cohen, who have been overseeing this project, plan to check out our unit, the prototype of a unique 4-ring design, at the Scandatronix factory in Sweden prior to shipment. We have contributed significantly to the NIH acquisition of the cyclotron needed to efficiently utilize the PET scanner, which is also due to arrive here this Spring. We have similarly helped to finance the Clinical Center's Nuclear Magnetic Resonance scanning facility. I have initiated negotiations with the NIH Nuclear Medicine and Radiology Departments to insure an equitable distribution of both PET and NMR resources, based on each Institute's individual contributions.

Several new positions and other resources will also be required to create our planned Laboratory of Molecular Genetics. As I mentioned last year, this new field offers an unprecedented opportunity to advance our understanding of the brain. Similarly, initiatives in the area of behavioral medicine -- beyond just providing psychiatric consultation to NIH Institutes -- could become possible next year if the budget provides new resources.

This year, our Intramural Program has become eligible to participate in the Research Service Act fellowship program designed to train young physicians who might not otherwise pursue research careers.

A whole cast of current and former Intramural scientists -- as well as a number of our patients -- were featured on public television for eight weeks this Fall as part of the excellent WNET Brain Series, which was funded in part by NIMH. These include Drs. Mort Mishkin, Tom O'Donohue, Norm Rosenthal, Tom Wehr, Lew Bigelow, Frank Putnam and Danny Weinberger. A major portion of the program on "Madness" was filmed at our Saint Elizabeths Program.

The possibility of a major change in that Program surfaced over the past year, with word that Dr. Erminio Costa has been asked to head a proposed new neuroscience institute at Georgetown University. Fidia, Inc., a non-profit U.S. affiliate of Fidia Pharmaceuticals, Italy, would be underwriting this joint venture, which could also attract several other Laboratory of Preclinical Pharmacology scientists. Meanwhile, Congress this year passed legislation setting a timetable for the transfer of Saint Elizabeths Hospital to the District of Columbia by 1989, giving further imminence to decisions regarding the future of our clinical program at the Hospital. Prospects for relocating parts of our Saint Elizabeths programs on the NIH campus dimmed this year when planning funds were stricken from the budget for construction of a new building on the NIH campus. Among several options now under consideration is the possibility of NIMH leasing space in the proposed Fidia building at Georgetown. Dr. Norton Neff ended a long and productive career at the LPP this year to become professor and chairman of Pharmacology at Ohio State University.

Dr. Richard Wyatt's Neuropsychiatry (formerly Adult Psychiatry) Branch at Saint Elizabeths has just undergone a review by our Board of Scientific Counselors at this writing. Being able to tap the Board's expertise has been especially helpful at this time as important decisions concerning that program's future must be addressed.

Over the past year, the Board reported on its review of the methodologically sophisticated Laboratory of Socioenvironmental studies and the new Laboratory of Cell Biology -- the latter being largely a prospective review. The Board's report on the Clinical Neuropharmacology Branch (now within the Laboratory of Clinical Science) cited the remarkable amount of work accomplished with limited resources.

Last year, the Board awarded the orphaned Clinical Infant Research Program at Adelphi high marks as a prevention services research and demonstration effort but found it out of place in Intramural Research. A more appropriate placement has now been arranged within the Health Resources and Services Administration, Division of Maternal and Child Health. The Institute has developed a cooperative agreement with HRSA to ease this transition and we wish Dr. Stan Greenspan and colleagues well in this new organizational arrangement.

Joining the Board as new members this year are: Dr. Daniel Freedman, UCLA Neuropsychiatric Institute, Dr Herbert Weissbach, Roche Institute of Molecular Biology, Dr. Bernard Grosser, Univ. of Utah Dept. of Psychiatry and Dr. Arthur Prange, Univ. of North Carolina Dept. of of Psychiatry. Our ongoing Board members -- whose combined institutional memory provides critical continuity -- are: Drs. Norman Garmezy, Anita Hendrickson, and Robert Moore. Dr. Brenda Milner's term on the Board ended this year.

Leaving Building 9 and retiring from Federal service this year is our long-time veterinarian Dr. Jere Phillips. Jere plans to continue ministering to research monkeys in the private sector. We welcome his successor Dr. Bob Werner to NIMH. Bob has long been associated with the NIH.

Lengthy negotiations with ADAMHA personnel have led to the appointment of Joseph Whitaker as head of the personnel office for the Intramural Program. Joe, who also comes to us from NIH, is a highly competent and dedicated professional with an understanding of the proper role of personnel in the development and maintenance of a high quality research enterprise.

To close on a personal note, it's been a challenging but satisfying year to be Director of Intramural Research. Internally, we have settled down appreciably from the earlier reorganizations. Although the prolonged uncertainty of the leadership transitions above us was at times distracting, the outcome was indeed a fortunate one. With strong leadership again in place above, I look forward to devoting my full energies to the Intramural Program with a little bit left over for my own scholarly pursuits.

ANNUAL REPORT OF THE BIOLOGICAL PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1983 - September 30, 1984
Robert M. Post, M.D., Chief

The Biological Psychiatry Branch consists of several Section/Unit level groups of investigators participating in clinical, research, and associated basic science projects. Dr. Thomas W. Uhde serves in a dual role as Chief of the 3-West Clinical Research Unit for the study of mood and anxiety disorders, and as the director of an extensive outpatient clinical evaluation and treatment unit for patients with generalized anxiety and panic anxiety disorders. Dr. Philip Gold is Chief of the Unit on Clinical Neuroendocrinology which is being converted to a Section. He is engaged in the study of neuroendocrine alterations in a variety of neuropsychiatric conditions as well as evaluating behavioral alterations in primary endocrine disorders in order to assess potential underlying pathophysiological mechanisms. He has recently been recognized for his studies of CRF in affectively ill patients with the award of the C.P. Richter prize in Psychoneuroendocrinology. Dr. David Rubinow has a dual role as Chief of the NIMH Consultation Liaison Service for the NIH Clinical Center and as Chief of the Unit on Peptides within the Biological Psychiatry Branch. In both of these roles he is actively engaged in the study of the relationship of endocrine and peptide alterations to those of mood, behavior, and cognition. Dr. Agu Pert is Chief of the Unit on Behavioral Pharmacology which is engaged in the development of clinically pertinent animal models of behavioral disorders, in the study of the mechanism of action of psychotropic drugs, and in classical neurotransmitter-receptor and neuropeptide interactions. Dr. Paul Marangos is Chief of the Unit on Neurochemistry which is engaged in the study of basic mechanisms of neuroenzyme and receptor regulation and their potential clinical implications. Dr. Jitendra Patel is studying the neural and molecular aspects of protein phosphorylation as it relates to basic mechanisms of neural transduction and to alterations in responsivity and behavior in clinically relevant models.

The work of the Section on Psychogenetics is summarized at the end of this annual report as this group, headed by Dr. Gershon, was elevated to the Branch level during the year.

Unit on Anxiety and Affective Disorders (Dr. Thomas W. Uhde, Chief)

This Unit investigates the phenomenology, physiology, biochemistry, and pharmacology of patients with primary anxiety disorders. Special attention is given to patients with panic disorder and its associated agoraphobia as it occurs in isolation and in association with major affective illness. The pattern of panic disorder has been carefully characterized in 38 patients in which there is great variation in the frequency and pattern of distribution of panic attacks within and among individuals. Eighty-four percent of these patients present with moderate to severe agoraphobia. This avoidant behavior developed after, rather than before, the first panic attack in 97% of the cases. Fifty percent of the patients had a lifetime incident of severe depression but in only half of these was the duration of the depression greater than two weeks. These data are of particular interest in relationship to common and divergent treatment stategies for major affective illness and primary panic disorders. Both appear to be responsive to tricyclic and monoamine oxidase inhibitor treatments. In parallel, benzodiazepines are not considered a drug of choice either for primary depression or panic disorder (in which generalized or anticipatory anxiety is responsive to benzodiazepines but these agents are not considered drugs of choice for the panic attacks themselves).

These clinical data provide a most interesting background for investigation of common and differential biological correlates of affective illness and panic-anxiety disorder. For example, Dr. Uhde has found that patients with panic-anxiety disorder have a significantly shorter REM latency than age- and sex-matched normal volunteers, but the decrease is not of a similar magnitude to that observed in primary depression. Moreover, instead of increases in REM density observed in affectively ill patients, populations of panic-anxiety patients have decreases in REM density. These patients also have increased muscle movement during sleep. Panic patients have blunted growth hormone response to the relatively selective neuroadrenergic alpha-2 agonist clonidine similar to that observed in depressed patients. They also have platelet beta receptor binding characteristics and PGE_1-stimulated cyclic AMP production similar to depressed patients. In contrast to depressed patients, they do not show a high incidence of cortisol hypersecretion, as revealed by escape from dexamethasone suppression.

Dr. Uhde has demonstrated possible noradrenergic involvement of panic anxious patients with his observations that clonidine has anxiolytic properties in both depressed and anxious patients. In contrast, yohimbine, an alpha-2 antagonist which increases the firing of the noradrenergic locus coeruleus, is associated with marked increases in anxiety in panic patients, but not in normal volunteers. These data both suggest that alterations in noradrenergic mechanisms may be associated with alterations in anxiety and that panic-anxious patients may have an increased responsivity to the alpha-2 agonist yohimbine.

Work with a caffeine questionnaire suggests that panic-anxious patients may also be more responsive to caffeine than either affectively ill or normal volunteer control populations. Studies with caffeine challenges have revealed that caffeine produces dose-related increases in anxiety with the highest doses inducing panic attacks in association with marked increases in cortisol in two normal volunteers. Preliminary data suggest panic anxious patients may be more responsive to caffeine challenges consistent with the caffeine questionnaire data and the fact the more panic anxious patients have given up consumption of caffeine containing beverages (i.e. coffee) than matched control populations. The mechanisms mediating the caffeine-induced changes, particularly those relating to adenosine receptor systems, are being investigated.

Work in Dr. Uhde's group has also demonstrated that the induction of hypoglycemic symptoms in conjunction with a five hour glucose tolerance test is associated with increases in anxiety but not the induction of panic attacks in these panic predisposed patients. These data indicate that there is pharmacological selectivity in the induction of panic attacks and that panic anxious patients are not nonspecifically responsive to any pharmacological challenge which induces autonomic instability, particularly one that mimics many aspects of their anxiety syndrome. Ongoing double-blind clinical trials are evaluating the clinical and biological effects of alprazolam, clonidine, imipramine, and carbamazepine in the treatment of generalized anxiety and panic attacks. Preliminary data suggests that each of these medications may be useful in some patients included under this diagnostic rubric. A primary goal of the Unit on Anxiety and Affective Disorders is to identify possible clinical and biological markers of differential pharmacological responsivity. It is hoped that assessment of baseline biochemical alterations and those induced by various pharmacological challenges may help elucidate subgroups with common mechanisms and pharmacological responsivity.

The Unit on Clinical Neuroendocrinology (Dr. Philip Gold, Chief)

This Unit, which has been elevated to a Section level, is engaged in preclinical and clinical studies of neuroendocrine function, particularly in relation to the hypothalamic-pituitary-adrenal axis. Dr. Gold's major focus this year has been on corticotropin releasing factor (CRF) a peptide shown in experimental animals to be a principle endogenous stimulus to ACTH secretion. It was unknown, however, whether CRF was of physiological relevance to human HPA function,, and thus of potential interest in studies of depression, Cushing's disease, and other serious illnesses. To explore CRF's relevance to human physiology, Dr. Gold and his colleagues first studied primates and normal volunteers, comparing the biological effects and pharmacokinetics of ovine CRF (oCRF) and the more recently sequenced human CRF (hCRF). Dr. Gold and his colleagues demonstrated that both peptides have equal ACTH releasing potency, but that the metabolic clearance rate of hCRF was ten times greater than for oCRF. In light of hCRF's short half-life, Dr. Gold used this ligand to indirectly explore the possible relationship between endogenous CRF and pulsatile ACTH secretion. It was noted that a single hCRF pulse given to volunteers produced an ACTH response which, in duration and amplitude, closely resembled naturally occurring ACTH pulses. Moreover, when patients with hypothalamic CRF deficiency were given hCRF pulses administered strategically during the 24 hour period (i.e., timed to mimic the temporal organization of naturally occurring ACTH pulses), a normal secretory pattern of both ACTH and cortisol ensued. These studies suggest that hCRF plays a physiological role in pulsatile ACTH secretion in man and heighten interest in exploring the possible role of CRF in clinical disorders characterized by abnormal ACTH secretion.

Although the long half-life of oCRF makes it unsuitable for studying ultradian ACTH secretion, this ligand, which functions as a long-acting analog of hCRF, has been helpful as a diagnostic agent and in exploring the pathophysiology of hypercortisolism in various disease states. For instance, depressed patients have high basal cortisol levels and smaller ACTH and cortisol responses to exogenous CRF than controls, suggesting that the pituitary in depression is restrained by the negative feedback effect of the elevated cortisol levels. In contrast, patients with Cushing's disease have markedly exaggerated ACTH responses to CRF despite their high baseline cortisol, indicating impairment of cortisol negative feedback upon the pituitary. These data indicate that testing with oCRF may help distinguish depression from Cushing's disease. In addition, they are consistent with a central nervous system-hypothalamic (CRF) mechanism for the hypercortisolism of depression and a pituitary mechanism for the hypercortisolism in Cushing's disease. In support of this formulation are observations from Dr. Gold's studies in normals, which show that a continuous oCRF infusion produces a picture of hypercortisolism very similar to that classically seen in depression, while a continuous ACTH infusion produces the kind of hypercortisolism most typically seen in Cushing's disease.

The oCRF test has also proven helpful in the differential diagnosis of secondary adrenal insufficiency and between Cushing's disease and ectopic ACTH secretion. Moreover, in hypercortisolism psychiatric patients with diagnoses other than depression (i.e., anorexia nervosa, primary anxiety disorders) the pattern of ACTH responses to oCRF is similar to that seen in depression. In light of the available data suggestive of a hypothalamic defect which results in the hypersecretion of CRF in hypercortisolemic psychiatric disorders, Dr. Gold and his colleagues have conducted basic studies on the regulation of CRF secretion in several species of non-human primates as well as in intracerebroventricularly cannulated sheep.

Dr. Gold's clinical studies with corticotropin releasing factor in collaboration with Dr. G.P. Chrousos were recognized in his award of the C.P. Richter Prize in Psychoneuroendocrinology.

Dr. Gold is also studying oxytocin and vasopressin in plasma and cerebrospinal fluid of neuropsychiatric patients and their associated behavioral and cognitive correlates. In many learning and behavioral paradigms, oxytocin and vasopressin have opposing functions. Thus, it is of interest to find reciprocal alterations in these substances in cerebrospinal fluid of manic and depressed patients. Where non-psychotic bipolar depressed patients tend to have lower CSF vasopressin than manic patients, the opposite is true for oxytocin. Reciprocal alterations in these hypothalamic peptide hormones have also been observed in the CSF of patients with anorexia nervosa.

The Unit on Peptides and NIMH Consultation Liaison Service (Dr. David Rubinow, Chief)

As Chief of the Consultation Liaison Service, Dr. Rubinow has initiated a unique series of interdisciplinary studies in collaboration with various NIH Institutes to study interactions of medical and psychiatric disorders. For example, in collaboration with Dr. R. Joffe he has observed a high incidence of depression associated with cancer of the pancreas but not in association with gastric cancer. In addition he has noted that this is associated with cortisol dysregulation as revealed by escape from dexamethasone suppression. A large series of other projects are being spearheaded by this service and by clinical associates specifically assigned to work intensively with a given clinical research unit and Institute. These collaborative projects should encourage increasing interaction among NIMH and the other Institutes, as well as being clinically and scientifically productive.

In his work with the Unit on Peptides on the 3-West Clinical Research Unit, Dr. Rubinow has found that CSF somatostatin is significantly reduced in depressed patients and normalizes with improvement and the switch to mania. These findings have now been replicated by five other investigative groups. He has also found that the anticonvulsant carbamazepine, which is also effective in the treatment of patients with manic-depressive illness, significantly decreased somatostatin in cerebrospinal fluid. These observations are of interest in light of recent data by other investigators indicating that kindled seizures produce long-lasting increases in brain somatostatin and that depletion of brain somatostatin with cysteamine is associated with anticonvulsant effects on amygdala kindling. Thus, it is possible that either the psychotropic or the anticonvulsant effects of carbamazepine may be associated with alterations in somatostatin function. Dr. Rubinow has also found that carbamazepine produces cortisol hypersecretion, as evidenced by increases in 24-hour urinary excretion of free cortisol in those with initially normal basal levels, and it produces consistent escape from dexamethasone suppression. It is possible that the effects of carbamazepine on somatostatin, i.e. decreasing its inhibitory control of the hypothalamic-pituitary-adrenal axis, accounts for the cortisol hypersecretion during carbamazepine administration. Consistent with this formulation are the observations in depressed and schizophrenic patients that Dr. Rubinow has made in collaboration with Drs. Doran, Pickar, Roy, and Paul. They found that patients who showed abnormal escape from dexamethasone suppression in both diagnostic groups had significantly lower levels of somatostatin in their spinal fluid. Thus, it is possible that decreases in somatostatin function as reflected in levels in CSF may be a correlate of the abnormal pattern of cortisol hypersecretion that has been consistently revealed in studies of depressed patients in the international community over the past decade.

Dr. Rubinow is also investigating possible biochemical and anatomical correlates of the cognitive impairment that is observed in depressed patients. He found that depressed patients with higher levels of excretion of urinary free cortisol showed greater defects on the Halstead Category Test. Moreover, there was preliminary evidence that patients with increased ventricular brain ratio (studied in collaboration with Dr. C. Kellner) also showed greater impairment on the Category Test. It was particularly of interest to find that depressed patients with higher levels of cortisol excretion showed increased ventricular brain ratio on their CAT scan. These preliminary data suggesting an interrelationship between cortisol hypersecretion, CAT scan abnormalities, and cognitive defects have been recently replicated by Dr. V. Reus and colleagues who demonstrated that those patients who escaped from dexamethasone suppression showed increased ventricular brain ratios as well as greater cognitive impairment on neuropsychiatric test batteries. These finding provide the basis for further dissection of the causal links in this relationship and a clarification of the relationships of cortisol hypersecretion, cognitive defects and anatomical changes in brain in patients with other neuropsychiatric syndromes such as Cushing's disease and anorexia-bulimia.

Dr. Rubinow has completed diagnostic screening in a large series of patients with reported menstrually-related mood disorders. He has critically reviewed the literature in this area and offered several methodological suggestions for the study and clarification of this syndrome. He has found that approximately 50% of patients self referred for severe menstrually-related mood disorders do not meet strict criteria for such a syndrome and may have mood fluctuations that do not vary closely with menstrual phase. Prospective and longitudinal evaluation of these two patient populations should lead to more rapid advances in dissecting underlying biological mechanisms of the syndrome and in elucidating new pharmacological treatments. Double-blind clinical evaluations of pyridoxine, progesterone, carbamazepine, and methoxy-progesterone are currently in progress. Dr. Rubinow has screened over 500 women for this project and is analyzing extensive questionnaire data. Neuroendocrine, peptide, psychological, physiological, and associated biochemical indices are being studied in both symptomatic and asymptomatic phases of the cycle.

Unit on Behavioral Pharmacology (Dr. Agu Pert, Chief)

Dr. Pert's work focuses on the interaction of classical neurotransmitter and neuropeptide effects on behavior, response to acute and repeated stress, and the elucidation of the mechanism of action of clinically important drugs such as lithium and carbamazepine. Dr. P. Clarke in his group has developed techniques for autoradiographic visualization of nicotine receptors labelled with either ^{3}H-nicotine or ^{3}H-acetylcholine in the presence of atropine. Both of these ligands revealed the same highly discrete pattern of receptors different from those labelled by the more commonly used nicotinic probe, ^{125}I-alphabungarotoxin. These nicotinic receptors were seen in high concentration in thalamic nuclei, interpeduncular nucleus, superior colliculus, substantia nigra, molecular layer of the the dentate gyrus, presubiculum, and lamina 3 and 4 of the cerebral cortex. In contrast, ^{125}I-alpha-bungarotoxin binding revealed an entirely different pattern with highest binding in cerebral cortex, hypothalamus, hippocampus, inferior colliculus, and certain brain stem nuclei. These findings suggest the presence in brain of two distinct subtypes of nicotine receptors. Lesions of the habenula decrease the binding of ^{3}H-nicotine in the interpeduncular nucleus; this implies that some binding sites may be "autoreceptors". Lesions of the median forebrain bundle decrease binding of ^{3}H-nicotine in the zona compacta of the substantia nigra, suggesting the presence of nicotine receptors on dopamine cell bodies. Systemic injections of nicotine increase the firing rate of dopaminergic compacta neurons in the substantia nigra, also suggesting that nicotine can influence dopaminergic neural activity.

Injections of phencyclidine (PCP), a major drug of abuse, into the nucleus accumbens produced locomotor excitation which was blocked by systemic administration of haloperidol, while injections of PCP into the periaqueductal gray in mesencephalic reticular formation produced decreased locomotor output.

Calcitonin was found to produce potent analgesic effects following injections into the periaqueductal gray and mesencephalic reticular formation.

Electrostimulation of the arcuate nucleus is found to produce a naloxone-reversible analgesic effect that was accompanied by decreases in binding in ^{3}H-diphenorphine in terminal regions of the beta endorphin system suggesting the release of endorphins by this manipulation. Techniques for studying in vivo release of opiate substances have been developed by Dr. T. Seeger in collaboration with Dr. C. Pert. A variety of stresses have been shown to decrease ^{3}H-diprenorphine binding (suggestive of receptor occupancy by endogenously released substances) in many selective subcortical structures, while the cortex and hippocampus were largely unchanged. The greatest decreases were seen in brain areas such as periaqueductal gray, reticular formation, and the midline-intralaminar nuclei of the thalamus. Compared to the changes produced by cold water stress, foot-shock stress produced a similar pattern but less selective decreases in ^{3}H-diprenorphine binding in amygdala and nucleus accumbens, suggesting a relatively selective release of opiate substances in these areas during amygdala kindled seizures. Similar changes were not observed following electroconvulsive seizures.

Utilizing 2-deoxyglucose for studying regional glucose utilization, Seeger and colleagues observed differential patterns of metabolic activity in animals who were administering brain stimulation reward in comparison to animals that received similar parameters of stimulation administered by the experimenter. Moreover, divergent and convergent patterns of glucose utilization were observed following rewarding brain stimulation of the ventral tegmental area compared to substantia nigra. Similar changes in glucose utilization were found in nucleus accumbens and prefrontal cortex while divergent patterns were observed in the terminal fields of the substantia nigra and tegmental area.

Studies of the behavioral and environmental consequences of unavoidable compared to avoidable stress have initiated in a collaboration with Dr. S. Weiss. In this paradigm it is hoped that psychological constructs such as the ability of animals to cope with stress, compared to yoked animals who are unable to control similar degrees of stress, should provide an important model for studying clinically relevant behavior and concomitant biochemical alterations which may be similar to those observed in depression and anxiety disorders.

Unit on Neurochemistry (Dr. Paul Marangos, Chief)

A major area of investigation involves the study of adenosine receptors in the central nervous system. Adenosine uptake sites have been identified in dog heart, and it has been demonstrated that calcium antagonists nimodipine and nifedipine interact with these sites. Extensive data document that adenosine uptake sites and receptor sites are distinct. The uptake sites do not respond to caffeine and have a distinct pharmacology and anatomical pattern of distribution. Chronic treatment with caffeine has been shown to increase adenosine receptor sites. Moreover, the caffeine-induced increases in the adenosine receptors are seen in rat pups whose nursing mothers were receiving caffeine. These data indicate that the caffeine content in mother's milk may be sufficient to affect brain adenosine receptors in offspring.

The anticonvulsant and psychotropic drug carbamazepine is a competitive inhibitor of both agonist and antagonist adenosine binding while it does not affect the uptake site. Carbamazepine is ten-fold more potent as an inhibitor of antagonist compared to agonist binding. Chronic administration of carbamazepine produces an upregulation of the adenosine receptor, suggesting the possibility that it acts as an adenosine antagonist.

Solubilization and purification of both the receptor and uptake site is in the initial phases of work. Dr. Marangos is currently preparing an affinity column in conjunction with John Daly's group and this team hopes to be able to separate the receptor and uptake site, purify each, and raise antibodies against each.

Dr. Marangos has also conducted pioneering studies in basic aspects of neuron specific enolase (NSE) which, additionally, are now of major clinical interest and import. An expanded series of patients with small cell lung cancer indicates that serum NSE levels can predict tumor relapse up to 12 weeks before conventional chest X-rays show tumor relapse. This biochemical marker derived from basic laboratory studies is expected to have a major clinical impact in the treatment of this neuroendocrine cancer. In the area of pediatric neuroblastoma, Dr. Marangos has also shown that 98% of patients had elevated NSE levels that correlated with clinical course. Thus, NSE levels in serum appear to be of major importance in the diagnosis and management of two major neuroendocrine neoplasms. Preliminary results also suggest that stroke patients may show increases in NSE levels in serum which may become a sensitive means for characterizing an ischemic episode and may ultimately be of clinically diagnostic value.

The basic studies of NSE are focused on cloning the gene for NSE from rat brain. These studies are being conducted in collaboration with Dr. M. Brownstein and investigators at the Salk Institute. Rat NSE is currently being sequenced so that a DNA probe can be made to extract the gene. A major target of this research will be the investigation of the process of the switch from NNE to NSE that Dr. Marangos showed to occur during neuronal differentiation.

Dr. J. Patel's work focuses on the study of brain protein phosphorylation which appears to be a critical link in the biochemical events transducing neurotransmitter-receptor interactions into final physiological responses. A variety of evidence suggests that protein phosphorylation plays a critical role in converting primary and secondary messenger responses into physiologically important neural signals. In collaboration with Robert Rebois, Dr. Patel has found preliminary evidence that receptor desensitization may involve a process of protein phosphorylation. In murine leydig tumor cells they found that binding of HCG to its receptors stimulates cyclic AMP production and causes the system to become refractory to further stimulation by hormone. Phorbolester, a cancer producing agent, produced desensitization indistinguishable from that produced by hormone. The actions of phorbol ester are mediated by the C-kinase suggesting that such desensitization might involve phosphorylation of one of the regulatory components of the C-kinase. By exposing HCG antibodies to solubilized HCG receptors, Dr. Patel has been able to precipitate the HCG receptor and show that it becomes phosphorylated when exposed to hormone.

In contrast to mechanisms of desensitization, amygdala kindling represents a sensitization process in which the animal becomes increasingly responsive to the same intermittent stimulus over time. Receptor changes associated with amygdala kindling have not been consistently delineated in spite of repeated attempts in many laboratories. Dr. Patel has found that electrical kindling of the amygdala results in an increased phosphorylation of a specific brain membrane protein of 35,000 molecular weight. This change in phosphorylation was found in both the left and right amygdala of kindled animals suggesting that it was not an electrode artifact, and did not occur in animals receiving electroconvulsive shock further suggesting it was not a nonspecific concomitant of generalized seizures. These findings have now been extended in a second series revealing that S100 modulated phosphoprotein (SMP), synapsin, and the 35 K (previously reported as 45 K) protein are phosphorylated in the left amygdala membrane preparations of kindled animals but not in sham stimulated or prekindled (stimulated only to the presence of after discharges) controls. Further studies are indicated in order to confirm whether these findings are associated with the long-term changes associated with kindling and to further elucidate the molecular mechanisms involved.

Studies are also progressing in the characterization and elucidation of the functional role of a unique brain phosphoprotein whose phosphorylation is inhibited by S100 and by calmodulin. This protein, named S100 modulated phosphoprotein (SMP), is present in both brain supernatent and membrane. A brain extracted growth factor called neurite extension factor (NEF) was found to convert SMP from a 87 K molecular form to a 90 K form. Further studies are warranted to demonstrate whether the mechanisms of NEF in stimulating the growth of neurites involves changes in phosphorylation.

Section on Psychobiology (Dr. Robert M. Post, Acting Chief)

A major focus of the work of this Section, in collaboration with Dr. T.W. Uhde, continues to involve delineation of the efficacy and biochemical mechanisms of action of the anticonvulsant carbamazepine in manic-depressive illness. Our ongoing double-blind clinical trials documents acute and prophylactic efficacy of carbamazepine in manic and depressive phases. Ten of 17 acutely manic patients have shown an excellent response. Twenty of 37 acutely depressed patients have shown a moderate to good antidepressant response. We have followed nine treatment refractory patients for an average of 2.7 years on carbamazepine. Using a mirror image analysis we have found substantial reduction in the frequency of both manic and depressive episodes during carbamazepine treatment. A major effort now involves the identification of possible clinical and biological markers of carbamazepine response. We have observed better response to carbamazepine in those who are initially more severely depressed or manic, in those who show a positive antidepressant response to one night's sleep deprivation, in those who show a pattern of more rapid cycling illness, and in those who do not respond to lithium treatment. Preliminary evidence suggested that the patients who showed better responses had higher levels of the active metabolite carbamazepine-10,11-epoxide, while levels of carbamazepine itself were not closely correlated with degree of clinical response. A particularly interesting finding of clinical relevance was elucidated by Drs. P. Roy-Byrne and R. Joffe. They found that carbamazepine decreased levels of T3 and T4 and that, surprisingly, those who responded best to carbamazepine had greater decreases in T4 and free T4 compared to non-responders. Dr. Joffe then examined the relationship of lithium response to thyroid indices and found that patients with evidence of greater impairment of thyroid function, as indicated by large increases in TSH on lithium, responded better to this agent. He found preliminary evidence that ECT was also associated with decreases in thyroid function. These data appeared inconsistent with those in the literature indicating T3 potentiation of antidepressant response. However, he noted that T3 "potentiation" reduces circulating levels of T4, and, since the brain preferentially utilizes T4 for intercellular conversion to T3, such a treatment might also be associated with relative decreases in T4 levels and its functional consequences in brain. Thus, investigation of a potential side effect of carbamazepine has led to a reformulation of the relationship of alterations in thyroid indices to degree of antidepressant response to the major treatment modalities for affective illness including lithium carbonate, ECT, and carbamazepine.

Substantial progress has been made in elucidating neurotransmitter and peptide effects of carbamazepine, although to date none of the multitude of effects produced by carbamazepine has been definitively linked to its psychotropic properties. However, preliminary evidence suggests that alterations in noradrenergic function may be associated with its anticonvulsant effects. New data, in collaboration with Dr. S. Weiss, also indicate that the anticonvulsant effects of carbamazepine on amygdala kindling are different from those of diazepam. Using agents with some selectivity for actions at "central"and "peripheral" benzodiazepine receptors, she found that the anticonvulsant effects of diazepam were reversed by Ro15-1788 but not by Ro5-4864. In contrast the anticonvulsant effects of carbamazepine were blocked by the "peripheral" ligand Ro5-4864 but not by Ro15-1788, a relatively pure inactive antagonist of the "central" benzodiazepine receptor site. These data suggest important new avenues of investigation of the possible differential molecular mechanisms involved in the effects of carbamazepine in contrast to diazepam.

As noted above, carbamazepine decreases somatostatin in the CSF of our affectively ill patients in contrast to other psychotropic agents studied. Preliminary evidence suggests that carbamazepine, in contrast to lithium carbonate, produces vasopressin agonist-like effects on renal function, findings which may be of clinical and theoretical import. In addition, a substantial body of evidence indicates that carbamazepine is not inducing its antimanic effects through blockade of dopamine receptors, as has been postulated for the action of classical neuroleptic agents. As such carbamazepine may have clinically important effects in affectively ill patients without carrying the liability for producing acute Parkinsonian side effects and the more problematic side effects of tardive dyskinesia. As indicated above, carbamazepine exerts potent effects at adenosine receptors which may be related to its profile of clinical effects or side effects.

Clinical trials of two other anticonvulsants, phenytoin and valproic acid, are also underway in order to evaluate specificity of the psychotropic response to anticonvulsants in patients with manic-depressive illness. Preliminary evidence indicates that patients may show differential response to carbamazepine and valproic acid. It is hoped that comparing and contrasting the mechanisms of action of carbamazepine to other anticonvulsants, and to more traditional psychotropic agents such as lithium, neuroleptics, and tricyclic antidepressants, will reveal important biochemical and physiological mechanisms involved in its efficacy in manic-depressive illness.

Dr. C. Kellner is completing studies of intravenous procaine administration as a potential probe of limbic system responsivity. Data in animals had suggested that local anesthetics such as lidocaine and procaine exert relatively selective electrophysiological and metabolic effects of libmic system structures. Preliminary confirmation of these findings are derived from work in collaboration with Dr. R. Coppola and in collaboration with a team of investigators including Drs. R. Cowdry, D. Gardner, F. Putnam, B. Vittone, P. Gold and J. Chassey. Procaine administration induces a selective pattern of increases in fast frequencies on the EEG particularly 45 Hz over the temporal cortex. This is associated with increases in frontal slowing and an obliteration of parietal and occipital alpha activity during procaine. The selective activation of fast activity over the temporal lobe is consistent with the hypothesized selectivity of procaine for limbic structures and their associated temporal lobe connection.

These physiological responses are associated with alterations in mood and cognitive function, as well as increases in secretion of ACTH, cortisol, and prolactin, but not growth hormone. Preliminary evidence suggests that utilization of this pharmacological probe and its associated mood, cognitive, endocrine, and electrophysiological effects may differentiate patients with borderline personality disorders from affectively ill subjects and normal controls. We also hope to study whether acute responses to procaine will predict subsequent pharmacological response to agents such as carbamazepine, which have important effects on temporal lobe and limbic substrates.

Dr. S. Weiss has inititated a series of studies examining the effects of corticotropin releasing factor (CRF) on seizures and behavior. She found that intracerebroventricular administration of CRF produced the late onset of seizures which physiologically and behaviorally resembled those produced by amygdala kindling. Repeated administration of CRF was associated with apparent tolerance to CRF-induced seizures yet produced sensitization to subsequent electrical kindling of the amygdala. Marked increases in irritability and aggression were observed during CRF administration. These findings suggest the possilibity that CRF in pathological circumstances may alter convulsive and behavioral responsivity in a long lasting fashion. Studies are ongoing in attempts to identify biochemical and molecular mechanisms involved in long term changes of amygdala kindling. In addition, Dr. Weiss has initiated behavioral and biochemical studies of the effects of avoidable and unavoidable stress, a paradigm which has been used as a model of depression and anxiety.

In the office of the Chief, Dr. G. Brown has continued his studies of the relationship of biochemical variables, particularly low levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5HIAA) to aggession and suicide. His data in patients with personality disorders indicate that those with low levels of 5HIAA have higher incidence of aggression and suicidality. In contrast, in a cohort of affectively ill patients studied by Dr. Roy-Byrne, a relationship between 5HIAA and suicidality was not observed. Within the personality disorder populations studied by Dr. Brown he has identified a clinical picture of mood volatility, lack of guilt, assaultiveness, hostility, and irritability in those individuals with low CSF 5HIAA. He plans to extend this series of studies to include patients with extremely violent behavior, and studies on the first patient in this series have been completed.

Dr. Frank W. Putnam, who is now with the Adult Psychiatry Branch, continues his studies of patients with multiple personality syndrome that were initiated on the 3-West Clinical Research Unit. He has documented important behavioral, autonomic, electrophysiological, and cognitive alterations across different personalities within the same patient in subjects with the multiple personality syndrome.

Section on Psychogenetics (Dr. Elliot Gershon, Chief)

The Section on Psychogenetics has as its goals (1) identification of biological and psychological factors that are genetically transmitted in major psychiatric disorders and (2) pharmacologic and pharmacogenetic studies of psychoactive agents in patients with major psychiatric illness and in normal controls.

Affective Disorders: This Section continues to pursue the hypothesis of abnormal cholinergic function as a genetic trait marker for familial affective illness. In fibroblasts, additional patients and relatives showed the predicted association of increased cholinergic receptor density with affective disorder and decreased density with normal psychiatric status. Studies to purify and characterize the muscarinic receptor protein in these cells are under way. In a new series of bipolar patients and controls, an attempt is being made at replication of the previous finding that patients show greater sensitivity than controls to cholinergic induction of rapid eye movement (REM) sleep. Preliminary results are positive.

New fibroblast and lymphoblast cell lines have been established from members of three large affective disorders pedigrees.

In a diagnostic study of school-age (6-17) offspring of bipolars, compared with appropriate age-matched controls, a modest, but statistically significant, increase in children with any psychiatric diagnosis, but no specific increase in affective diagnoses, was found.

The "high-risk" method of investigating putative trait markers for psychiatric disorders has been analyzed statistically. Based on this analysis, a long-term biological and psychological follow-up study has been initiated, studying adolescent and young adult offspring of bipolar and appropriate age-matched controls. Cell lines have been collected from these individuals.

CSF studies in euthymic bipolar outpatients revealed no major differences from controls in 11 neuropeptides or in GABA, or various monoamines or their metabolites. Two-dimensional gels of CSF proteins also showed no differences.

cDNA probes for several neuropeptides and proteins of interest are being studied for restriction fragment length polymorphisms, by hybridization (Southern blots) to genomic DNA from patients and controls.

Schizophrenia: Family studies and biologic-genetic studies of schizophrenia have been started.

Alzheimer's disease: Twenty-two new protein polymorphisms defined by two-dimensional electrophoresis were studied in a large pedigree with Alzheimer's disease. Linkage to the disease was not found. Two of the polymorphisms were found to be identical to the classical linkage markers PGM3 and GLOI.

Annual Report of the Clinical Neuropharmacology Branch
National Institute of Mental Health
October 1, 1983 to September 30, 1984

Dennis L. Murphy, M.D., Chief

The Clinical Neuropharmacology Branch's projects in the last year ranged from phenomenological characterization of patients with various neuropsychiatric disorders to developmental work using biochemical and physical science methodologies to approach biomedical problems. These projects all have a common goal: an increase in understanding of the mechanisms of action of drugs which are or may prove to be effective in treating psychiatric disorders, and, of course, clarification of the nature of the disorders themselves.

The clinical studies are primarily based on our clinical research unit which is temporarily housed on the 6D NIH Clinical Center Ward. Some outpatient studies are carried out in the ACRF clinics opened last year as well as in the protocol and one-way mirror rooms on the 6D unit; the 6D protocol rooms are better equipped for certain of our procedures, and also permit more economical use of staff, as well as avoiding duplication of less mobile instrumentation for similar inpatient and outpatient studies. Laboratory studies are carried out in our two-module biochemical pharmacology lab on the 3 North corridor. Rodent studies use a shared module on the 4C corridor, and non-human primate studies are accomplished using a portion of the common DIRP primate laboratory space in Building 9.

A major new venture launched this year which involved our Branch was the development of a joint NIMH-NICHD project at the Poolesville animal facility for the study of normal and psychopathological developmental processes in non-human primates. Dr. Thomas Insel has been named to head a new unit on Comparative Brain and Behavior in the Clinical Neuropharmacology Branch to develop both his own line of research and, more particularly, to collaborate with Dr. Steven Suomi, who holds joint appointments as Chief of the NICHD Laboratory of Comparative Ethology and Assistant Director for Research on Primate Behavior in the NIMH-DIRP. Dr. Suomi is bringing a large rhesus monkey colony from the University of Wisconsin to a newly established outdoor corral area at the NIMH-NICHD Poolesville facility.

Other general changes in the Branch in the last year included: (a) the necessary diminution of new project development in the area of obsessive-compulsive disorder research which Dr. Insel had been directing before he took over his new non-human primate project responsibilities; (b) a larger emphasis on both inpatient and outpatient work on the affective components and neuropharmacologic aspects of patients with dementias, sparked by a confluence of Dr. Robert Cohen's leadership in this area (which had been gathering impetus before he was appointed to head the Clinical Brain Imaging Section in the Laboratory of Psychology and Psychopharmacology last year) together with the interests of Drs. Trey Sunderland, Pierre Tariot and Herbert Weingartner in cognitive assessment, neuropharmacologic challenge testing and experimental treatment approaches in this disease; and (c) the "graduation" from the Branch at the end of the year of four long-term staff members, Dr. Jonathan Costa, Dr. Carol Hoover, Mr. Joseph Aloi, and Mrs. Irene Bellesky, all of whom will be much missed; and (d) my own assumption of some additional, intramural program-wide responsibilities as Associate Director for Clinical Studies. Fortunately, the Branch currently includes an excellent contingent of remaining junior staff and several highly promising new staff; we also continue to benefit from the extremely able direct and collaborative continuing contributions from Drs. Robert Cohen, Larry Siever, and others, so that the Branch's traditional level of high productivity and innovative work has been only minimally affected.

In projects related to the affective disorders and drugs used in their treatment, especially the selective monoamine oxidase (MAO) inhibitors, several advances can be highlighted. Studies in depressed patients designed to explore neurotransmitter-neuroendocrine relationships revealed evidence of an altered association between cortisol and norepinephrine function in depressed patients compared to controls. Using the selective alpha-2 adrenoreceptor agonist, clonidine, a significantly greater reduction in plasma cortisol was found in the patients--a finding which may prove important in linking long-postulated norepinephrine system abnormalities in depression with the abundant evidence of cortisol hypersecretion and dexamethasone suppression subsensitivity in depression. In contrast to these findings regarding cortisol--where abnormalities prexist in depression--other physiologic functions, including plasma MHPG and heart rate reductions, were less responsive to clonidine in depressed patients compared to controls. These latter findings were similar to previous observations from our group and others regarding a dampened growth hormone response to clonidine. Taken together, these data continue to support aberrations of alpha-2 adrenergic regulation of brain neurotransmitter and neuroendocrine function in depression.

Investigations in depressed patients of central serotonin system function also revealed reduced prolactin responses to the selective presynaptic releaser of serotonin, fenfluramine, in a large general sample of depressed patients compared to controls, as well as in a smaller sample of patients carefully age- and sex-matched with controls. Similar reductions in prolactin responses to the serotonin precursor, l-tryptophan, were found by other research groups this year, strengthening the likelihood that this is a replicable abnormality in these patients. Continuing work with this neurotransmitter-neuroendocrine paradigm in the coming year includes examination of the cortisol response to fenfluramine and comparative studies initiated by Dr. Edward Mueller using the direct serotonin receptor agonist, meta-chlorophenylpiperazine. Our pilot studies with this latter compound in rhesus monkeys showed sharp elevations in prolactin, cortisol and growth hormone over a six-fold dose range (0.5-3.0 mg/kg). Although this agent has not previously not been studied in man, we obtained an IND for its use on the basis of its being a metabolite of the recently marketed antidepressant, trazadone, and on the evidence from our monkey and rodent studies.

In rodents treated on a long-term basis with the selective monoamine oxidase type A-inhibiting antidepressant, clorgyline, adaptive response alterations were found to meta-chlorophenylpiperazine and to clonidine by Dr. Channa Aulakh. The reductions in responsivity to meta-chlorophenylpiperazine in regard to food intake and locomotor activity appear to represent behavioral correlates of brain serotonin pathway adaptations found in several studies of brain serotonin receptors during chronic but not acute treatment with effective antidepressant drugs. Similar reductions in locomotor activity responses to clonidine after 21 but not 7 days of treatment with clorgyline appear to represent behavioral correlates of the reductions in brain alpha-2 adrenoreceptors found in the same animals. While both noradrenergic and serotonergic neurotransmitter systems were found to be prominently altered during chronic clorgyline administration to rodents, comparative studies in rhesus monkeys yielded results more similar to those obtained in limited studies in man which indicated that noradrenergic system changes--particularly MHPG reductions in cerebrospinal fluid, plasma and urine--were more prominent than serotonergic system changes. Because more extensive studies involving cerebrospinal fluid are possible in non-human primates, we are pursuing these observations using challenge tests with meta-chlorophenylpiperazine and clonidine in combination with pretreatment with inhibitors and stimulators of neurotransmitter synthesis, and with other selective inhibitors of MAO-type A (amiflamine, cimoxatone) and of MAO-type B (deprenyl).

In electrophysiologic studies, clorgyline was found to slow the firing rate of locus ceruleus neurons in rodent brain. This alteration precedes beta- and alpha-2 adrenergic receptor downregulation, and is thought to represent an intermediate stage in the adaptive events following MAO-inhibitor treatment. Although we and others have described brain receptor changes in rats, and cerebrospinal fluid amine metabolite changes in monkeys following clorgyline, no change in platelet alpha-2 adrenoreceptors or in the cyclic AMP response to norepinephrine was observed in depressed patients receiving clorgyline chronically. As we have also observed reduced blood pressure responses to clonidine in depressed patients receiving clorgyline, a finding suggestive of a functional alpha-2 adrenergic down-regulatory response in man, the lack of change in platelet receptors and cyclic AMP responsiveness in the human platelet studies suggests that this cell may not provide a reliable or sensitive model for receptor changes in the brain following antidepressant drug treatment.

In projects related to other areas of psychopathology, recent studies defining cognitive function abnormalities in depression have been extended to explorations of brain mechanisms which may contribute to the known cognitive impairment found in several other neuropsychiatric syndromes. A close link had been demonstrated last year between motivational state and memory performance in depressed patients. To examine this linkage further, ongoing studies of the effects of the opiate system antagonist, naloxone, in normal subjects and patients with early senile dementia of the Alzheimer type were performed. Other groups have reported preliminary studies suggesting a beneficial effect of naloxone in Alzheimer patients, but minimal effects in normals. Our initial studies in normals and depressed patients using a wider dose range of the drug than investigated previously indicated that doses of 2 mg/kg or higher in normals increased depression and anxiety and produced decrements in memory performance. Adequate cognitive data is not yet available from the patient groups, but preliminary impressions suggest that both depressed and Alzheimer patients may be more behaviorally sensitive to lower doses of naloxone, with activating rather than depressant effects predominating. The cognitive effects of cholinergic agents and monoamine oxidase-inhibiting antidepressants are also currently being studied in Alzheimer patients.

In continuing investigations of the clinical phenomenology and treatment of patients with obsessive-compulsive disorder, over 200 patients and relatives have now been studied by Drs. Insel, Hoover, Mueller and their coworkers. A double-blind, crossover comparison of the therapeutic effects of the tricyclic antidepressant clomipramine versus clorgyline revealed consistent improvement on measures of obsessionality, depression, and anxiety after four and six weeks of clomipramine treatment, but no significant changes after clorgyline. Improvement with clomipramine was not limited to patients with secondary depression.

There was a significant correlation between improvement in obsessional symptoms and plasma levels of clomipramine (but not with clomipramine's metabolites), and also with changes induced by clomipramine in psychophysiologic responses to aversive stimuli. As clomipramine (but not its metabolites) is a selective inhibitor of serotonin uptake, other measures reflective of serotonin function have been examined in the last year. Although the data are still being analyzed, the initial results do not indicate that significant patient-control differences exist for platelet [^{3}H]-imipramine binding or platelet serotonin uptake nor for cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations. Similarly, fenfluramine-induced plasma prolactin responses appear not to differ in the patients. Therapeutic responses to another tricyclic antidepressant, desipramine, which has more prominent noradrenergic than serotonergic effects, were negligible, while clomipramine administration again proved effective, suggesting that further examination of the properties of clomipramine compared to other tricyclics might be useful in trying to understand its therapeutic effects in obsessive-compulsive disorder. Among a large sample of family members of patients with obsessive-compulsive disorder, general personality characterisitics of super-cleanliness and over-meticulousness were prominent. Although 12% of the relatives had developed various psychiatric disorders, no other cases of symptomatic obsessive-compulsive disorder meeting diagnostic criteria were found.

In explorations attempting to develop new models in animals for psychopathologic states in man, the benzodiazepine receptor active antagonist beta-carboline-3-carboxylic acid-ethyl ester has been studied in rhesus monkeys. Dr. Insel and his coworkers found that this compound produced a dose-dependent behavioral activation in monkeys, accomplished by increased heart rate and plasma cortisol concentrations. These effects proved to be antagonized by the benzodiazepine anti-anxiety agent diazepam, but generally not by catecholamine-active drugs such as propanolol or clonidine. Although cortisol hypersecretion is a prominent hormonal alteration accompanying increased anxiety or depression, the administration of corticotropin-releasing factor intraventricularly in monkeys did not have a behavioral activating effect nor a catecholamine elevating action, although the expected changes in plasma cortisol did occur. Receptors for corticotropin- releasing factor were found to be widely distributed in the brain, suggesting that it may have other functions besides its role in regulating cortisol release.

Model systems for exploring monoamine storage, uptake, and metabolism in less complex tissue than brain continue to be evaluated by Dr. Costa for their possible help in understanding drug effects and physiologic processes relevant to psychiatric disorders. Human platelets as well as various eukaryotic cells have been studied using biochemical techniques as well as fluorescence microscopy, electron and proton imaging techniques, and nuclear magnetic resonance. Prominent effects of tricyclic antidepressants were found on a proton-pumping ATPase of lower eukaryocytes; these drugs also uncouple mitochondrial oxidative phosphorylate in human platelets. Serotonergic mechanisms in human platelets have been clarified using ^{3}H-serotonin and quinacrine as probes. Storage of serotonin in membrane-bound vesicles (dense bodies) of platelets has been proposed to occur either as a result of the formation of macromolecular complexes between nucleotides and serotonin, or because of the existence of an electrochemical proton gradient across the vesicle membrane. Tests of the applicability of these hypotheses to serotonin storage in the dense bodies of human platelets have been made by examining the disposition of quinacrine and serotonin in these organelles following varying treatments. The binding now seems less likely, since solid analogues of the dense body core (calcium, adenine nucleotides, and pyrophosphate) were not found to significantly bind serotonin or quinacrine. Incubation of platelets with substances which disrupt the electrochemical gradient released a large percentage of the intra-platelet quinacrine. A much smaller fraction of the total platelet serotonin was released by similar treatment, suggesting that the electrochemical gradient may not be required for serotonin storage. Because inhibition of the *de novo* uptake of serotonin into dense bodies failed to cause a significant loss of the serotonin stored in this compartment, serotonin stores do not appear to be maintained by active serotonin uptake. Several substances which enter the dense bodies equally well at 0°C and 37°C caused release of serotonin at 37°C but not at 0°C. The release observed at 37°C thus cannot be attributable to collapse of the electrochemical gradient or to the displacement of serotonin from intra-granular binding sites, but may be related to increased membrane permeability to serotonin at 37°C. Based on these observations, it appears as if serotonin taken up into the dense bodies of human platelets is retained because the dense body membrane has a very low passive permeability for serotonin; many compounds which cause serotonin release at 37°C may act by increasing this permeability.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Thomas A. Wehr, M.D., Chief
October 1, 1983 through September 30, 1984

The Clinical Psychobiology Branch directs its primary research effort towards understanding causes of affective disorders (depression and manic-depressive illness) and sleep disorders, and towards developing new forms of treatment for these disorders. In seeking causative mechanisms we have sometimes found it useful to study cyclic or regularly recurring forms of affective illness, such as rapid-cycling manic-depressive illness, seasonal affective disorder (winter depression) and premenstrual depression. In such patients affective episodes can be studied longitudinally and prospectively, and inferences and hypotheses about causative mechanisms can be drawn from sequences of concomitant biological and behavioral changes. Wherever possible we have attempted to test such hypotheses by experimental interventions designed to alter clinical state by altering biological processes. For example through such experiments we have determined that wakefulness late in the night, but not early in the night, results in clinical improvement in depression, and that exposure to bright artificial light ameliorates seasonal depression, possibly by suppressing secretion of melatonin by the pineal gland. Future experiments are planned in order to determine whether therapeutic alterations in sleep schedules act by stimulation of the hypothalamic-pituitary-thyroid axis (as descriptive, correlative studies suggest) and to confirm whether phototherapy in winter depression depends on the time of day of light administration (photoperiodism) and suppression of nocturnal melatonin secretion. The practical value of such experiments is obvious. Both light and sleep modifications act rapidly (1-3 days) to abolish depression. Both experiments are of great theoretical interest inasmuch as they provide novel foci for further investigations of brain mechanisms underlying clinical change in depression. In seasonal depression the retina appears to be the gateway to neural pathways involved in seasonal mood changes, and in depression in general processes occurring during sleep in the second half of the night are strongly implicated as causative mechanisms. We have attempted to pursue these clues in a systematic fashion employing experimental tests of hypotheses wherever possible. For seasonal affective disorder we have been fortunate to have been guided by extensive basic research in animal models. Knowledge of neural and endocrine systems involved in photoperiodically controlled seasonal behavior in animals led us to investigate these systems in patients. At the present time evidence implicating the retino-hypothalmic-pineal axis and melatonin secretion in seasonal depression is moderately compelling, and will be sought in future studies. The experimental alterations of sleep schedules which have powerful effects on depression also powerfully affect neuroendocrine function. For example, wakefulness in the second half of the night strongly stimulates hypothalamic-pituitary-thyroid axis hormone secretion and virtually abolishes pituitary prolactin secretion. Experiments involving analogous direct neuroendocrine perturbations in the absence of sleep deprivation will be explored in order to test whether these symptoms are involved in the responses to sleep perturbations.

The timing and amount of sleeping and waking as well as seasonal responses to changing light conditions are both regulated by a clock-like pacemaker in the hypothalamus, the suprachiasmatic nucleus (SCN). In its generation and regulation of daily and seasonal rhythms the SCN is strongly dependent for its synchronization with corresponding cycles in the natural environment on input from the retina via the retino-hypothalamic tract (RHT). Thus much of our research has been inspired by findings in basic research concerning the RHT and SCN, and has involved extending these findings to normal and aberrant human functioning.

In the sections that follow individual investigators provide overviews and progress reports in the various areas of investigation in the branch.

Seasonal Affective Disorder

Norman E. Rosenthal, M.D.

The Seasonality Study began approximately four years ago when we became aware of a few patients, who had a history of regularly occurring winter depressions separated by periods of euthymia or hypomania in the spring and summer. These patients attracted our attention because of our long-standing interest in rhythmic mood processes and the psychobiology of light. An extensive animal literature in seasonal (circannual) rhythms of behavior suggested that these patients might be undergoing an analogous process. Seasonal rhythms in animals are influenced by changes in the physical environment especially daylength (photoperiod). Photoperiodic information is conveyed from the eye to the suprachiasmatic nuclei (SCN) of the hypothalamus via the retino-hypothalamic tract. From there nerve impulses pass via the superior cervical ganglion to the pineal gland. The hormone, melatonin is secreted by the pineal gland at night in a circadian rhythm generated by the SCN. Environmental light suppresses nocturnal secretion of melatonin, which serves as a chemical transducer of photoperiodic, and therefore seasonal, information. Shortly before we encountered our first patients with winter depression, Lewy and others in our branch had shown that in humans the nocturnal secretion of melatonin could be suppressed by light of high intensity (2500 lux) but not by regular indoor lighting (approximately 500 lux). This suggested to us that patients with winter depressions might have circannual rhythms synchronized by photoperiod but that only when light was above a certain threshold intensity could it influence the system. This hypothesis could be tested by extending the winter photoperiod with high intensity environmental light in patients with SAD and observing the effect on mood.

After one successful case study in the winter of '80-'81, in which a patient responded well to bright light treatment, we were encouraged to seek out other patients with a similar seasonal problem and did so by means of an article in a local newspaper. We received several thousand responses to the article, suggesting that mood and energy difficulties in the winter are by no means uncommon. A systematic evaluation of the responses revealed at least one characteristic syndrome of seasonally occurring mood changes, which we have termed Seasonal Affective Disorder (SAD). Patients with this condition are predominantly women with an onset of symptoms in their twenties. In the D.C. area symptoms generally begin around October and last until March or April. Patients complain of fatigue, oversleeping, increased appetite, carbohydrate craving, weight gain and sadness. They have difficulty concentrating and work performance generally suffers. They withdraw from friends and family and interest in sex is greatly reduced. Generally depressions are mild to moderate in severity and hospitalization is rarely necessary. Approximately a third of the patients in our program have never been treated for the condition before. There is frequently a history of sensitivity to a series of overcast days, of improvement after travelling south in the winter and deterioration after returning north again. Summers are frequently characterized by hypomania and patients mostly meet criteria for bipolar II affective disorder.

In nine patients with SAD we evaluated the effects of extending the photoperiod for three hours before dawn and three hours after dusk with 2500 lux full-spectrum light for two weeks and used dim (100 lux) yellow light as a control. Patients had a marked antidepressant response to the bright light, which occurred within two to four days, and relapsed following withdrawal of lights within the same time frame. No such response was apparent with the control condition. Although this was encouraging, we were aware that there were at least two possible explanations for this result apart from the psychoactive properties of light, namely a placebo effect and sleep deprivation, which occurred to a significantly greater degree under bright than under dim conditions.

The following year we set out to replicate our findings and address ambiguities about whether the light itself was active. We recruited a new population of patients with SAD and found the clinical and demographic features of the patients to be remarkably similar from the first to the second year. We treated two separate groups of patients in a cross-over design: six as inpatients and seven as outpatients. Both groups had bright (2500 lux) light as the active condition. Morning and evening hours were again used but treatment was given for only one week. Control treatments were very dim light (<5 lux) for inpatients and 300 lux full-spectrum light for outpatients. In both groups we demonstrated a statistically superior effect of bright light. There was an equal amount of sleep deprivation under both treatment conditions, indicating that sleep deprivation was not a sufficient explanation for the antidepressant effects of our intervention. However, since there was a statistically significant difference between the amount of sleep recorded under baseline and both treatment conditions, we could not rule out the possibility that some degree of sleep deprivation was necessary for the antidepressant effects of light to occur.

We followed up this question in our most recent winter study ('83-'84) when we again recruited a new series of patients and treated nine patients for a week with five hours of light in the evenings only. We used 2500 lux and 300 lux of full spectrum light in a cross-over design. Patients were encouraged to maintain their normal sleep schedules and no sleep deprivation occurred under either treatment condition. We again found that bright light had significant antidepressant effects and dim light did not. This result suggests that sleep deprivation is not necessary for the antidepressant effects of light treatment. When the results of the four studies from the three years were compared, no difference was found between groups. This suggests that light treatment administered in the evening only is as effective as combined morning and evening treatment and that the early morning hours believed to be critical for eliciting an antidepressant response to sleep deprivation are not similarly critical for light treatment.

The problem of whether light treatment may be working by means of a placebo effect has not been completely resolved to date. However, mounting evidence suggests that this is unlikely as a sole explanation for all the observed results. The results of the four studies on the effects of bright environmental light are very similar with regard to degree and timing of improvement. The pattern of relapse following withdrawal of light is also similar from year to year. Patients generally improve or relapse within two to four days and not immediately as one might expect if a placebo effect were operating. Treatment with bright light on subsequent occasions is almost invariably followed by a response similar to the initial response. Patients have used the lights throughout the winter months without any loss of efficacy, which is also unusual for a placebo effect. A variety of control lighting conditions have been used with varying degrees of plausibility as an active treatment modality. Yet the overall results have been consistently negative.

The best placebo response was noted in the most recent study with evening light treatment. This is counterintuitive as one would assume that the most recent group of patients would have had maximum exposure to publicity about light treatment and the hypotheses underlying treatment design and would therefore have the strongest psychological bias against the control treatment. In certain cases there seems to have been a therapeutic reaction to the control light especially when 300 lux have been used. In these cases it is possible that the light intensity has been above the therapeutic threshold and the effect may therefore be related to the light itself although, of course, a placebo explanation cannot be excluded. It is important to realize that perception of brightness is variable and depends in part on the brightness of an individual's environment prior to observing the light in question. In our experience both patients and staff have at times confused the 2500 and 300 lux lights.

It is probable that the issue of placebo effects of light cannot be definitively addressed in any individual study since it can always be argued that subjects had some psychological reaction to the light which critically influenced their response. It is more likely that a growing body of work on an increasing number of patients at a variety of centers will make a placebo explanation seem increasingly less probable. One study involving light manipulations which we are planning for the coming winter and may address this issue in a novel way involves the use of "skeleton photoperiods". In animal studies two pulses of light administered at the extremes of a given time interval, a "skeleton photoperiod", will be registered by that animal as an unbroken photoperiod of that interval. In our new study we are planning to administer "long" and "short" skeleton photoperiods to patients with SAD. This should provide us with further information about the formal mechanisms by means of which light treatment works, perhaps enabling further comparisons with the animal literature. In addition, since we would not predict that patients would have *a priori* expectations that a short skeleton photoperiod should have an effect different from a long one, the actual amount of light used being equal under both conditions, a different response to different skeleton photoperiods would argue further against a placebo effect.

We have measured certain biological correlates of SAD during both winter and summer phases in an attempt to understand the pathophysiological basis of the condition. EEG sleep studies performed on 17 patients showed increased sleep length, increased REM density and decreased delta sleep during the winter months. Comparable studies in eight normal volunteers did not show these seasonal changes. Dexamethasone suppression tests performed on 20 patients showed normal cortisol suppression and infusions of TRH in seven subjects showed normal TSH responses. Recently Larry Tamarkin has joined our branch, bringing with him a sensitive and specific RIA for melatonin. Preliminary studies in four SAD patients show that nocturnal melatonin secretion does not appear reduced as has been described for other depressed populations and may actually be elevated. In addition patients appear to have abnormal spikes of melatonin secretion during the day. If this finding is borne out when larger numbers of patients are studied it may prove to be a marker for SAD and perhaps even causally related to the symptoms.

There are other reasons for considering melatonin as a possible mediator of some of the symptoms of SAD. Melatonin is of ubiquitous importance in other animals as a chemical transducer of photoperiodic information and a mediator of seasonal rhythms. Light which is bright enough to suppress melatonin has antidepressant effects in SAD, whereas light too dim to suppress melatonin does not. Extending the period of melatonin secretion in animals has been shown to duplicate the effects of extending darkness and extending the darkness has been shown to induce relapses in SAD. With this information in mind we treated eight SAD patients with oral melatonin in conjunction with light treatment to see whether this would reverse the therapeutic effects of light. We found that melatonin did in fact reinduce a variety of symptoms, which are characteristic of the condition, though patients still felt better than before light treatment. To further test the hypothesis that melatonin may be of pathogenic significance in the syndrome of SAD, we are planning to administer beta-blockers, which reduce melatonin secretion, to patients with SAD to see whether this will ameliorate their symptoms. If beta-blockers prove helpful in SAD, they may offer a convenient alternative to light therapy.

While continuing to study the psychobiology of SAD and the antidepressant effects of light, we are also planning to conduct epidemiological studies of SAD in different parts of the country. We are also providing support in various ways to investigators, who ar studying SAD and the therapeutic effects of light in Europe (Belgium and Switzerland), on the West Coast, and in Minnesota, New Hampshire and Alaska.

Circadian Rhythms and Affective Disorders

David A. Sack, M.D.

The possibility that circadian rhythms are involved in the pathophysiology of affective disorders was suggested by the cyclical nature of recurrences seen in some manic-depressive patients and the observation that certain core depressive symptoms are temporally distributed over the course of the day, for example during sleep, episodes of awakening occur in the early morning and depressed mood is worse in the morning than in the evening. In addition, when normal subjects are placed in conditions where all time cues have been eliminated, they exhibit changes in their sleep and activity which are similar to those which occur in manic patients. These naturalistic observations stimulated descriptive investigations of circadian rhythms in patients with affective disorders and led to the development of new treatments which apply principles of circadian biology to this population.

The human circadian system can be conceptualized as consisting of two interacting biological clocks or oscillators. The first is a strong oscillator which controls the rhythm for core body temperature, cortisol, and REM sleep, while the second, weaker oscillator controls the timing of waking and sleeping. Investigations by our laboratory and by others have shown that timing of rectal temperature is sometimes shifted earlier in depression and the amplitude of this rhythm is decreased. Depressed patients also show characteristic changes in their sleep such as shortened REM latencies and long first REM periods and these abnormalities have been interpreted to reflect a phase-advance of the circadian rhythm of REM sleep propensity. Similar changes in the timing of the circadian rhythms for cortisol, urinary potassium, urinary and plasma MHPG have been described and, we have hypothesized that the abnormally early timing of these circadian rhythms relative to the sleep-wake cycle may be central to the symptomatic expression of affective illness.

Circadian rhythm abnormalities have not been consistently observed in all studies. Heterogeneity in the populations studied, with respect to diagnoses and degree to which subjects were free of psychotropic medications may account for some of these differences. A more serious limitation of previous studies arises from the fact that the timing of biological clocks cannot be measured directly in humans. The observed rhythms in temperature, hormones, neurotransmitters and their metabolites, can be distorted by external influences (a process called masking), that do not reflect the behavior of the circadian pacemaker. Common sources of masking in clinical studies are diet, physical activity, sleep schedules, and environmental lighting. The failure to control adequately for masking in previous studies may have obscured differences between patients and normal controls. Similarly the differences which have been observed may reflect a systematic bias in masking, caused by symptoms occurring with depression, such as diminished physical activity, shortened and interrupted sleep, or inadequate nutritional status. In our present study we have attempted to eliminate the effects of masking by studying the circadian rhythms of depressed patients and normal controls in constant conditions of sleep, diet and physical activity. Subjects are restricted to bed and kept continuously awake for thirty hours. During this time they receive a liquid diet in equal hourly feedings. Measurements are obtained for rectal body temperature, activity, plasma hormones and neurotransmitter metabolites throughout the study.

Recently we have described a circadian rhythm for the dopamine metabolite, HVA, is the plasma of depressed patients using this methodology. The rhythm for HVA peaks during the night and declines progressively over the day. The nocturnal rise occurs independently of sleep but daytime values of HVA are substantially lower when diet and activity are controlled. In recent clinical trials the dopamine agonist bromocriptine has been shown to have significant antidepressant effects, and studies of sleep deprivation in animal models have shown behavioral supersensitivity to dopamine following selective REM sleep deprivation. A possible relationship between the circadian rhythm in HVA and the antidepressant effects of sleep deprivation will be examined in future studies.

Another focus of our circadian studies has been the regulation of hormones whose secretion may be abnormal in depression. Abnormalities in thyroid function have frequently been observed in depressed patients, specifically a blunted thyrotropin response to TRH, an elevated TSH in patients treated with lithium, and an antidepressant response to triiodothyronine when added to antidepressant medications. A circadian rhythm has been described for TSH in normals which peaks late in the evening shortly before sleep and is partially suppressed by sleep. We questioned whether an underlying abnormality in the circadian organization of the hypothalamic pituitary thyroid axis (H.P.T.) could account for the dysregulation of TSH seen in some depressed patients. Circadian profiles have been obtained with q 30 minute sampling in 8 rapid-cycling manic depressive patients and four age matched, sex matched controls. The nocturnal rise in TSH is lower in the depressed patients at baseline and sleep deprivation which characteristically augments the rise of TSH produces a much greater effect in normals than in the depressed patients. Sleep deprivation does raise the TSH in the patients to levels which approximate the normals at baseline and this normalization in TSH may in some way mediate the antidepressant response to sleep deprivation.

TREATMENT OF DEPRESSION BY MANIPULATION OF THE SLEEP-WAKE CYCLE

The importance of circadian rhythms in the pathogenesis depression is suggested by abundant clinical evidence that altering the timing of the sleep-wake cycle leads to dramatic improvement in depressive symptoms. Open clinical trials of total sleep deprivation, partial sleep deprivation in the second half of the night, and phase advance of the sleep-wake cycle, have been shown to have antidepressant effects, whereas sleep deprivation in the first half of the night is less effective. One common aspect of the phase advance treatment and partial sleep deprivation in the second half of the night is that the wake-up time is shifted earlier. If, as we have hypothesized, the timing of circadian rhythms occurs earlier relative to the sleep period in depressed patients than these two treatments would tend to normalize the relationship between the two rhythms. Sleep deprivation in the first half of the night would not have this effect.

Previous studies of sleep deprivation were hampered by a lack of a credible sham-control. In addition, many studies included patients who were receiving concomitant treatment with antidepressant medications. In order to test whether the timing of sleep is critical in the antidepressant effects of partial sleep deprivation, we have examined the relative efficacy of partial sleep deprivation in the first half of the night as compared to partial sleep deprivation in the second half of the night in a randomized crossover design. Sleep deprivation in the second half of the night was associated with a significant reduction in the severity of depression whereas sleep deprivation in the first half of the night was not. Patients who responded to sleep deprivation had short REM latencies and long first REM periods, when compared with non-responders, consistent with a phase-advance of the REM rhythm. The two sleep deprivation conditions also differed significantly on their effects on sleep.

Partial sleep deprivation in the second half of the night was associated with a 50 percent reduction in REM sleep when compared with baseline sleep whereas REM sleep was unchanged on the inactive condition, a finding consistent with reports of an antidepressant effect of selective REM sleep deprivation.

Our present study of sleep deprivation is designed to further explore the relationship between abnormalities in the underlying circadian rhythms and the sleep deprivation response. In addition we are examining ways in which to improve the clinical utility of sleep deprivation by extending the duration of the response. Preliminary evidence from our previous study suggests that the antidepressant response to partial sleep deprivation can be enhanced and prolonged by a second night of treatment. We are now assessing whether sustained remissions can be achieved by repeated partial sleep deprivations.

Another area of interest is the possible interaction between circadian and pharmacologic treatments. Lithium and monoamine oxidase inhibitors can delay the timing of circadian rhythms and this may be one mechanism for their therapeutic effects. Circadian manipulations achieve a similar result by advancing sleep relative to the other circadian rhythms. We were interested in whether there might be a synergistic relationship between pharmacotherapy and phase-advance treatment. We have examined the effects of a phase advance of the sleep-wake cycle in four patients who were refractory to treatment with antidepressants. Patients were placed on a 6 p.m. to 2 a.m. sleep schedule while their antidepressant medications were continued. Three of the four patients became euthymic within a week, while the fourth patient was moderately improved. The clinical improvement was maintained after patients had been returned to their normal sleep schedules, and remissions lasted from 12 weeks to one year. Previous responses to phase advance therapy were limited to the two or three weeks following the intervention. The sustained remissions seen in these patients suggests a synergistic effect between antidepressant medications and treatment with a phase advance of the sleep-wake cycle.

THEORETICAL CONSIDERATIONS

The circadian rhythm disturbances seen in affective illness could arise from abnormalities in (1) the mechanism that entrains the pacemaker(s) to zeitgebers (2) the pacemaker itself (3) coupling between pacemakers or (4) distal to the pacemaker in the expression of its rhythm. A major goal of our program is to systematically test these hypotheses in our affective disorder patients.

An abnormality in the entrainment process could result in rhythms which become desynchronized from one another, a phenomenon which has been observed clinically in certain rapid cycling manic-depressive patients. An abnormal sensitivity to an entraining stimulus could also alter the relative timing of two circadian oscillators. In most animals, including primates, light plays an important role in synchronizing circadian rhythms. Recently a similar role for light has been demonstrated in humans. Studies performed in our laboratory and by others have shown that manic-depressive patients are supersensitive to the effects of light on the secretion of melatonin, a hormone which is secreted predominantly at night and whose secretion is suppressed by light. This supersensitivity appears to be a trait marker since recovered manic depressives continue to show this abnormality. It is possible that these patients are also supersensitive to the entraining effects of light.

Several methodological problems limit the interpretation of these studies. First, no attempt was made to control for the prior exposure to light. Secondly, since the amount of light received during the study was not precisely controlled differences in exposure could have accounted for some of the differences observed. Before one can explore the neuroendocrine and neurochemical bases for the supersensitivity to light it is necessary to be able to administer precise and continuous amounts of light and to be able to detect how much of that light is received. We have developed a ganzfeld dome which will enable us to administer to subjects lighting of uniform intensity and to precisely manipulate the lighting source with respect to wavelength and intensity.

Light is capable of entraining circadian rhythms by shifting them earlier or later. The phase-shift induced depends to a great extent on the time at which the light pulse is administered. Species differ with respect to their phase responses to light and the phase shifting property of light can be quantified and summarized in a phase response curve (PRC). A human PRC has yet to be described, largely for technical reasons. In our laboratory we are attempting to quantify the phase shifting properties of light in normal subjects using a modified PRC design. In subsequent studies we will compare the phase responsiveness of depressed patients with normal controls to directly test whether an abnormal phase response to light can account for the circadian rhythm disturbances seen in these patients.

In order to test whether a circadian rhythm disturbance is the result of a change in the intrinsic property of the pacemaker of from an abnormality in the coupling of the pacemakers, subjects must be studied in conditions where the rhythms are observed free from external influences. Studies of normal subjects in temporal isolation conditions have been conducted without difficulty for many years. We have recently completed longitudinal studies of four affectively ill subjects living in isolation from external time cues, for three to four weeks. These experiments were undertaken in part to test predictions of the following hypotheses: 1) rapid cycling forms of manic-depressive illness are caused by internal desynchronization of two circadian subsystems one of which is no longer entrained to 24 hour zeitgebers. This model predicts that manic depressives who are not rapid cyclers will become rapid cyclers in temporal isolation if internal desynchronization occurs. 2) The phase advance of circadian rhythms occurs because the intrinsic (free-running) rhythm of their driving pacemaker is abnormally fast. The intrinsic period of the depressive's pacemaker should be abnormally short in temporal isolation. 3) Changes in the phase relationship between sleep and circadian rhythms which occur during temporal isolation studies should produce clinical state changes predicted by partial sleep deprivation and phase-advance treatments.

Although the results of these studies should be regarded as preliminary they allow certain conclusions to be made about specific circadian hypotheses. Specifically we found no evidence that rapid manic-depressive cycles result from internal desynchronization of circadian rhythms. One patient we studied who exhibited rapid switches from depression into mania throughout her study, remained internally synchronized throughout, whereas a second patient who did become internally desynchronized did not develop rapid cycles, despite a past history of rapid cycling. In addition we found no evidence of an abnormally short intrinsic rhythm in two of our depressed patients. A short intrinsic period was however observed in one manic patient in the study. Additional studies are needed to confirm these findings before broad conclusions can be made about this patient group.

Premenstrual Syndrome
Barbara L. Parry, M.D.

Cyclic forms of affective disorders predominate in women in the form of rapid cycling manic depressive illness (RCMDI) and seasonal affective disorder (SAD). PMS resembles RCMDI and SAD in that it is cyclic and it occurs in women and is characterized by sleep disturbance. A project in progress in the Branch is designed to explore this analogy by determining whether sleep EEG patterns exhibit cyclic changes in PMS and whether manipulations of the sleep-wake cycle ameliorate PMS symptoms. This latter possibility is particularly interesting because excessive prolactin secretion has been implicated in PMS and prolactin secretion is known to be sleep dependent. In contrast to major affective disorders in which sleep deprivation has limited clinical feasibility because symptoms often recur after a night of recovery sleep, in PMS repeated total or partial sleep deprivation offers the potential of a clinically feasible treatment modality because symptoms may last only a few days.

The occurrence of cyclic forms of affective affective disorders may overlap in some women. For example, a high proportion (80 - 90%) of women with SAD have PMS which also responds to light treatment. We identified a woman whose premenstrual symptoms had a seasonal cycle: she was virtually relieved of symptoms during the summer and developed severe suicidal depressions in association with shortening of the daylength in the winter. High intensity light treatment was effective in relieving her premenstrual depressions in the winter. This therapeutic effect was blocked by administering melatonin simultaneously with the light, and reinstituted by administering propranolol, a B blocker which inhibits the synthesis and release of melatonin from the pineal gland. By this strategy, the role of seasonally changing melatonin in PMS is being investigated.

Sleep Studies
Wallace B. Mendelson, M.D.

The Unit on Sleep Studies is comprised of two facilities: a clinical laboratory, and a basic science laboratory which studies both animal sleep and biochemistry. A major effort is placed on applying insights from one level to testing on the other. We will describe several examples of such movement between clinical and basic science studies.

CLINICAL PROGRAM

The clinical laboratory currently is involved with two main projects, dealing with insomnia and with circadian rhythms of temperature. In the insomnia study, 10 patients and age-matched controls undergo studies of sleep, core temperature, daytime sleepiness and mood. Sleep studies have indicated that there are very few objective differences in the sleep of the two groups, suggesting, perhaps that the complaint of insomnia may be related less to the EEG than to the perception of sleep and cognitive processes during sleep. Awakening studies on these subjects indicated that insomniacs perceived REM sleep to be a "lighter" stage of sleep compared to normals. It was also observed that insomniacs have a higher core temperature than controls, although the overall temperature curve is in phase. During the daytime a multiple sleep latency test indicated that insomniacs have difficulty sleeping, once again suggesting that their problem is not merely a lack of mechanical sleep. Cognitive testing indicated that they can learn new material as easily as controls, but have a great deal of difficulty with semantic memory (the ability to retrieve and use previously learned material). This observation is leading to a new study in which these cognitive findings are being refined, and studies of cognitive function during sleep are being performed.

The clinical laboratory is also vigorously examining the relationship between sleep and temperature in the "space suit" study. Although the presence of clear circadian rhythms of both sleep and temperature are well described, the relationship between the two is unclear. One way to determine whether one is driven by the other would be to hold one rhythm constant while observing the other. To this end, we have constructed a system for holding core temperature constant while performing sleep studies. In summary, this is done by use of a nylon suit in which are embedded tubes through which hot and cold water flows. Rectal temperature data is sent to a computer which controls the regulation of core temperature in the subjects. So far six subjects have been studied, and data is currently under analysis. Possible uses for this system might include experimental treatment of depression by altering the relation of the daily temperature nadir to sleep time, and possible use as a treatment for sleep apnea syndrome. Another facet of the clinical sleep laboratory is support for a variety of circadian rhythm studies in the CPB, as well as collaborative work with other investigators. Among examples of such work are studies of seasonal affective disorder patients, in which it was observed that there is a circannual rhythm of delta sleep, and partial sleep deprivation studies, in which it was found that the antidepressant effect of sleep deprivation involves a critical period of the 24 hour cycle between 2:00 AM and 8:00 AM. Collaborative studies include a project showing that panic-anxiety patients may have short REM latencies, adding to the growing weight of opinion that this finding is not unique to depression.

BASIC SCIENCE PROGRAM

A common theme in the animal laboratory in the last few years has been the mode of actions of hypnotics and anxiolytics. Much of this has centered on studies of the benzodiazepine receptor. By use of selective benzodiazepine (BZ) receptor blockers, we have demonstrated that the receptor complex does indeed mediate the hypnotic actions of these widely used agents. Among our findings have been the observations that several beta carbolines, compounds which bind to the receptor complex and have opposite effects from benzodiazepines, are potent agents for enhancing wakefulness. We have also demonstrated the stereospecific nature of the BZ receptor vis-à-vis sleep by showing that two enantiomeric benzodiazepines, the B_{10} compounds, have opposite effects--one induces sleep and one induces wakefulness. This raises the possibility that the B_{10}^- might be useful as a clinical analeptic which is as benign as benzodiazepines. The issue of how BZ receptor stimulation induces sleep has been pursued. We have shown that nifedipine, a calcium channel blocker, will prevent the hypnotic actions of flurazepam, suggesting that calcium channel flow mediates the hypnotic action of these compounds. In an in vitro model of calcium uptake into synaptosomes, we have demonstrated that nifedipine and nitrendipine block diazepam-induced calcium uptake, suggesting perhaps some parallelism between the physiology and biochemistry of the system. We are currently exploring the relation of barbiturates to the BZ receptor complex. (They are known to bind to the chloride ionophore portion of the complex.) Tentative evidence from our laboratory suggests that some agents which block the chloride ionophore may greatly reduce the mortality from large doses of pentobarbital in mice, indicating that we may be moving in the direction of developing a clinical treatment of barbiturate overdose. Another example of movement from the basic science lab to clinical work is a collaborative study in which we will be examining the effects on sleep of RO 15-1788 a compound which occupies the BZ recognition site.

Movement from the clinical lab to basic science is exemplified by our current work with growth hormone (GH). For several years we have examined the mechanisms by which GH secretion is regulated in humans, finding that very different mechanisms are involved during sleep and waking. Now by use of measures of ornithine decarboxylase (ODC) activity (a measure of cellular growth, and hence the effects of GH), we are examining whether exogenous GH given during waking is less effective than when given before sleep in rats. We are also examining the relationship of GH function and exercise. Our data indicates that exercise enhances GH stimulation of ODC. This suggests that exercise not only stimulates secretion of GH, but results in more efficient action of the secreted hormone.

Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health
Seymour S. Kety, M.D., Acting Chief
Sanford P. Markey, Deputy Acting Chief
October 1, 1983 - September 30, 1984

Introduction

The various Sections of the Laboratory of Clinical Science have continued to conduct independent programs of research. These programs often involve collaborations within the Laboratory and with investigators in other institutes or outside of the NIH. The major activities of the programs are directed toward defining mechanisms regulating the development and function of the nervous system, understanding the role of the nervous system in maintaining bodily functions internal environment and mental state and determining the molecular events underlying drug and hormonal actions. Clinical studies are designed to define abnormalities and explain mechanisms of disturbed brain or other neurological functions, thereby providing rational bases for approaches to therapy of neuropsychiatric disorders.

During the past year, there have been significant changes in laboratory leadership and a reassignment of several of the sections of the Laboratory of Clinical Science.

Dr. Seymour S. Kety has served as Acting Chief following the departure of Dr. Irwin J. Kopin. Dr. Sanford P. Markey assumed the responsibilities of laboratory administration for most of this reporting period.

Upon the retirement of Dr. Julius Axelrod, the Section on Pharmacology which he developed and led to international recognition was transferred to the Laboratory of Cell Biology, ending an association of 29 years with the Laboratory of Clinical Science.

Dr. Michael Ebert moved from NIMH to Vanderbilt University, and Dr. David C. Jimerson, M.D. has been appointed Chief, Section on Experimental Therapeutics.

Dr. William Z. Potter was appointed Chief of a new Section on Clinical Psychopharmacology.

The Section on Child Psychiatry, headed by Dr. Judith Rapoport, is being established as a new laboratory.

The Section on Medicine, previously headed by Dr. Kopin, has been transferred to the National Institute of Neurological and Communicative Disorders and Stroke.

Dr. Seymour S. Kety received two prestigious awards for studies of cerebral circulation and schizophrenia: he was the first recipient of Japan's Mihara Foundation Award for his work on circulation and metabolism of the brain and Germany's Golden Kraepelin Medal for his achievements in psychiatry.

The following summaries of the year's activities have been provided by the the Section Chiefs of the Laboratory:

SECTION OF PHARMACOLOGY

Julius Axelrod, Ph.D., Chief

Adrenocorticotropin Release:

The cellular mechanisms involved in adrenocorticotropin (ACTH) release were studied in a tumor cell line of the mouse anterior pituitary (AtT-20/D16-16). Forskolin, an effective activator of adenylate cyclase increases cAMP accumulation, cAMP-dependent protein kinase activity and ACTH release with equal potency. The phosphorylation of several endogenous proteins is altered by forskolin acting through cAMP-dependent protein kinase. These data support a role for cAMP as a second messenger in the receptor-mediated release of ACTH.

Somatostatin (SRIF) is a potent inhibitor of ACTH release from AtT-20 cells. This peptide blocks hormone and forskolin-stimulated cyclic AMP formation and ACTH release by activating a guanine nucleotide inhibitory protein (N_i). The proposal is supported by experiments employing pertussis toxin. This agent catalyzes the ADP-ribosylation of N_i in AtT-20 cell membranes. Pertussis toxin blocks the ability of SRIF to inhibit corticotropin releasing factor (CRF) and forskolin-stimulated cAMP accumulation and ACTH release by inactivating N_i.

SRIF receptors can become desensitized on AtT-20 cells. Pretreatment with SRIF reduces the subsequent ability of SRIF to inhibit CRF and forskolin-stimulated cAMP accumulation and ACTH. This desensitization may involve an uncoupling of N_i and SRIF receptors.

Peripheral and Central Catecholamines in Hypertension and Stress:

We studied the role of brain and peripheral catecholamines, and other biogenic amines in the central regulation of cardiovascular function and during the stress response. Adrenal catecholamine synthesis is under control of dietary sodium in genetic Dahl hypertensive rats and under central control from specific brain nuclei. One of these, the fastigial nucleus of the cerebellum, regulates the secretion of catecholamines from the adrenal gland. We have localized specific brain stem and hypothalamic nuclei which participate in neurogenic and salt sensitive hypertension. In addition, by the combination of RIA and HPLC methods, we studied the prostanoid profile in specific brain regions and pituitary lobes of the rat.

Protein Carboxyl Methylation: A Post Translational Modifier of Protein Function:

Enzymatic carboxyl methylation of proteins is a post translational modification which reduces negative changes and alters their secretory structure and properties. It is associated with exocytotic release. Enzyme activity is very high in the posterior pituitary gland, where it is associated with methylation of neurophysins, and in the brain. We have found at least three still unidentified proteins, in addition to neurophysins contained within axons of brain origin in the pituitary gland. Determination of enzyme activity in hypothalamic nuclei demonstrate high activity in the magnocellular nuclei associated with production of vasopressin, oxytocin and neurophysins.

Role of Neuropeptides in Neuroendocrine Regulation:

This project studies the interrelationships between several neuropeptides (vasopressin, angiotensin II, corticotropin releasing factor and somatostatin) and their role in neuroendocrine regulation. We use animal models of genetic diseases: the Brattleboro rat, which in its homozygous state cannot produce vasopressin, and the spontaneously hypertensive rat. A relationship between angiotensin II, somatostatin and vasopressin can clearly be shown in individual brain nuclei and pituitary gland of Brattleboro rats. Angiotension II selectively stimulates glucose utilization in the subfornical organ and the posterior pituitary. We developed new techniques for the localization of angiotensin II receptors in brain nuclei and peripheral organs. We have found that two pituitary neuropeptides are present in fibers originated in the brain: corticotropin-releasing factor (in posterior lobe and intermediate lobe) and somatostatin (in intermediate lobe only). Specific roles for both peptides are postulated in the intermediate pituitary lobe.

SECTION ON HISTOPHARMACOLOGY

David M. Jacobowitz, Ph.D., Chief

This section has been involved with studies of peptidergic, monoaminergic and cholinergic neurons in the central nervous system. Immunocytochemical and neurochemical characterization of peptide-containing nerves in the brain and periphery has been pursued. Histochemical and biochemical analyses are carried out at the level of discrete central nuclei and pathways in order to define the underlying basis for behavioral changes and human pathology.

A major emphasis into the study of brain proteins is underway. The aim of this program is to reveal unusual proteins contained in various discrete nuclei and regions of the brain. We hope to generate antisera from such proteins which can be used as immunological probes, recognizing particular types of neurons, synapses or other structures. Such cytochemical markers have great potential for identifying cell classes and neuronal circuits. A variety of studies have been completed and are underway.

Using two-dimensional gel electrophresis (2DE), we have constructed an atlas showing the location and relative concentration of a number of different proteins from 25 distinct neuroanatomical regions of the male rat brain. The proteins are stained with silver and quantified by computerized densitometry. This atlas is a first attempt at systematically classifying the molecular weight, charge (pI) and relative concentration of proteins present in a variety of regions of the rat brain.

We have embarked on a series of experiments designed to ascertain which proteins are regulated by the major neurotransmitter systems in the CNS. The effect of desmethylimipramine (DMI) and reserpine on proteins from the rat hippocampus and parietal cortex was assessed by 2DE. These results demonstrate that chronic, but not acute, administration of agents affecting noradrenergic reactivity also affects the concentration of specific proteins within the central nervous system, and are of interest in view of the known effects of these drugs on β-adrenergic receptors.

Since the nucleus of the tractus diagonalis (td) provides the major cholinergic input to both the hippocampus and the occipital cortex, we decided to electrolytically ablate the td and examine protein concentrations in these two brain areas using 2DE. Of the 140 proteins examined, only four were found to be altered in concentration in both the hippocampus and the occipital cortex after the td lesion. The results suggest that the concentration of these four proteins in brain may be regulated by the cholinergic input to these brain areas. The possibility exists that one or more of these proteins in the brain may be related to the muscarinic cholinergic receptor. The results of this study are clinically relevant to Alzheimer's disease.

A method has been developed for the incorporation of [^{35}S]-methionine into proteins from freshly dissected brain tissue. The results indicate this technique will prove useful in examining the uptake of radiolabeled amino acids into protein from discrete nuclei and other brain areas and there is considerable regional heterogeneity in the incorporation o[^{35}S]methionine into proteins by the different brain areas examined.

Brain tumor samples from 50 patients with varying tumor types were studied using two-dimensional gel electdrophoresis (2DE) with silver staining. In addition, 12 different samples of fresh-frozen normal human cerebral cortex, previously irradiated cortex and postmortem cortex were analyzed using computerized densitometry. There were significant quantitative differences between the three groups of "normal" human cortex, although the qualitative patterns were very similar from patient to patient. However, even more remarkable was the finding that histological tumor type was associated with its own fairly distinctive and reproducible protein pattern. These findings indicate that biochemical diagnosis and gradings of human brain tumors based on their protein fingerprints is a realistic possibility.

We continue our ongoing interest in mapping the localization of brain neuropeptides as antisera become available. Growth hormone releasing factor (GRF) was revealed in the rat brain using an antibody to synthetic human GRF as an immunohistochemical marker. Corticotropin releasing factor (CRF)-like immunoreactive neurons have been identified in the rat retina by immunohistochemical methods.

Treatment of newborn rats with capsaicin (CAP) was shown to cause a disappearance of CRF-like immunoreactive nerve fibers in the dorsal horn of the spinal cord, the spinal trigeminal nucleus and tract and the nucleus tractus solitarius, but not in the median eminence and the central amygdaloid nucleus. Since it is well known that CAP acts selectively on primary sensory neurons of the C-fiber type, it is suggested that CRF is also located in peripheral sensory neurons, representing a novel peptidergic neuronal system, possibly involved in the modulation of transmission of peripheral nociceptive impulses, which is different from the CAP resistant hypothalamo-infundibular CRF system.

We continue with our investigations of the interpeduncular nucleus (IPN) of the rat brain. Immunocytochemical studies of IPN reveal a wide variety of endogenous peptides and biogenic amines distributed in somata and processes in a topographic and heterogenous pattern. Immunofluorescent cell bodies and processes were distributed differentially among IPN subnuclei, revealing new features of its organization. Forebrain and hindbrain afferent neurons projecting to the IPN have been demonstrated by retrograde transport of a fluorescent dye microinjected into the IPN. Medial habenular cells having substance P immunofluorescence were found, thereby documenting the substance P cell bodies that project to the IPN. Another unusual, relatively unknown nucleus, the nucleus incertus, a circumscribed region overlying the dorsal tegmental nucleus was revealed. Many of these cells were found to contain leu-enkephalin.

SECTION ON MEDICINE
Irwin J. Kopin, Chief

The major thrust of the studies in the Section on Medicine has been to study the mechanisms regulating autonomic activity and blood pressure. Studies are performed in animals as well as humans. In animals, various lesions can be made and effects of drugs examined. Investigations in humans are directed towards evaluating peripheral sympathetic function and central aminergic activity in normals and in patients with disorders of autonomic activity. The studies in humans are collaborative efforts with the Section on Clinical Pharmacology, NINCDS, and with Dr. David Goldstein, NHLB. In this section only the studies in animals will be described.

Peripheral sympathetic neurons regulate blood pressure and heart rate as well well as a variety of other autonomic functions. It is difficult to study the functioning of these neurons because there are reflexes mediated by the baroceptors and other afferents which modulate sympathetic activity. The outflow of the sympathetic neurons can be stimulated directly in pithed rats, however, so that such reflexes are eliminated and the degree of activation can be experimentally controlled. Using this preparation, the effects of drugs, hormones, exposure to stress, etc., can be examined in normal and genetically deviant rats.

Using pithed rats we have shown that the mean concentration of 10-7 nmole/l of norepinephrine at vascular neuroeffector junctions evokes a 50 mmHg increase in blood pressure and that about 2/3 of norepinephrine released during sympathetic stimulation is removed by reuptake into the neuron with the remainder diffusing into the circulation. This preparation has also been used to examine the effects of phencyclidine (PCP) on peripheral norepinephrine release. We have shown also that leukotriene D_4, a constituent of slow reacting substance of anaphylaxis, reduces the stimulation induced release of norepinephrine but also markedly diminishes the pressor effects of a variety of vasoconstrictors (eg., vasopressin, angiotensin) presumably by interfering with vascular smooth muscle constriction.

A technique for examining directly the sympathetic neurons in humans is being developed for use with Positron Emission Tomography (PET) scanning. Fluorinated catecholamines derivatives were examined with the aim of developing false adrenergic transmitters which could be labelled with ^{18}F and used in studies of adrenergic activity, in vivo. 6-Fluorodopamine was demonstrated to be taken up into sympathetic neurons, converted by dopamine β-hydroxylase to 6-fluoronorepinephrine (6F-NE) which is stored and released by sympathetic nerve stimulation in the same manner as endogenous norepinephrine. Thus, 6F-NE is a false adrenergic transmitter which can be used as a valid tracer in studies of noradrenergic functions in humans. Fluoro-DOPA can be used to label dopamine stores in brain and, in fact, has advantages over DOPA since it is a poor substrate for catechol-o-methyltransferase which readily converts DOPA to methoxy-DOPA.

The cardiac output and its distribution to the various tissues has been examined in pithed rats before and during pressor responses to sympathetic stimulation and infused norepinephrine. Opposite effects of circulating vs. endogenously released norepinephrine may indicate differences in accessibility to α_1 receptors, different receptors mediating the responses. This appears most prominently in the splanchnic vascular bed. This technique is now being applied to intact animals to determine effect of drugs, anesthetics, etc. on distribution of cardiac output.

Intact animals regulate blood pressure by controlling activation of various pressor mechanisms which can be activated when baroceptors are stimulated. The pathways in the central nervous system, the neurotransmitters involved in these pathways, and the pressor mechanisms involved in maintenance of blood pressure have been studied using a variety of techniques.

Deafferentation of the Nucleus Tractus Solitarius (NTS) evokes striking increases in blood pressure and heart rate. This response is attended by marked increases in plasma levels of catecholamines and vasopressin.

The changes in levels of catecholamines and their metabolites in areas containing the cell bodies (A_1,A_2,NTS) and nerve terminals (paraventricular and periventricular nuclei, PVN and PEN, respectively) suggest that catecholamines released in the PVN from cells originating A_1 area of the brain stem may inhibit vasopressin release and that removal of this inhibition evokes the release of this pressor peptide. Vasopressinergic neurons may provide a feedback mechanism via the A_1 and NTS areas to modulate vasopressin release through release of catecholamines from these nerve terminsls in the PVN. These neurons are severed when the NTS is deafferented but are intact when a hypotensive drug is administered. Thus the effects of the lesion are more striking than the effects of drug-induced hypotension.

Direct application of putative neurotransmitter peptides or related agonists provides a means for examining the possible role for such substances in regulating cardiovascular function. Opiate peptides selective for mu, delta, or kappa receptors were injected by microtechniques into the anterior hypothalamus or septal areas of rats. The results of such studies indicate that mu receptor stimulation is most effective in evoking a sympathetic response and a pressor effect, but vasopressin is not released. Higher doses of the mu agonist also evoke vagal responses and thereby reverse the tachychardia but not the pressor response.

Hemorrhage evokes a compensatory release of vasopressin, activates the renin-angiotensin system, and evokes a sympatho-adrenal medullary response. Studies with ganglionic blocking agents, vasopressin antagonists, and in animals which are unable to form vasopressin, indicated that either an intact sympatho-adrenal system or vasopressin secretion are sufficient to maintain blood pressure after moderate hemorrhage, but the absence of both results in rapid deterioration and death.

Changes in receptor levels in tissues reflect altered activity of neurotransmitter and compensatory changes which modulate function. Repeated or continuous exposure to agonists may produce subsensitivity whereas blockade or inactivity of receptors may increase their levels. Continuous infusion of nalaxone increases the three types of opiate receptors and produces supersensitivity of depressor and bradycardiac effects, but not respiratory effects to morphine. Chronic blockade of peripheral dopamine receptors with domperidane (which does not cross the blood brain barrier) increases dopamine receptors in the striatum, possibly by indirect hormonal mechanisms which are currently being examined. The increase in dopamine receptors produced by haloperidol blockade can be prevented by insulin, presumably via its hypoglycemic effects.

SECTION ON ANALYTICAL BIOCHEMISTRY

Sanford P. Markey, Ph.D., Chief

This section conducts research on the development and application of analytical instrumentation to problems in neuropharmacology. During the past year, most of the research effort has been directed toward applications.

The metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound which induces a parkinsonian syndrome in primates, was studied. Radiolabelled MPTP (^{3}H and ^{14}C) was synthesized and administered to rats, guinea pigs and monkeys, and the pattern of its brain metabolism characterized. In the monkey, MPTP is converted in the brain to an oxidized pyridinium species, MPP$^+$, which is intraneuronally trapped with a half-life of 10 days. Radioautography of monkey brain demonstrated a high degree of regional localization of MPTP metabolite, with greatest concentrations in dopamine neuronal terminal regions. This pattern of distribution persisted for more than 20 days in the monkey. In contrast, both rat and guinea pig clear MPTP and its metabolites from the brain in a few hours. These differences in the pattern of metabolism may be responsble for the observed differences in neurotoxicity. Neither the rat nor the guinea pig suffer loss of neuronal cell bodies in the substantia nigra, a characteristic feature of the neurotoxicity in the primate. Presently, studies are focusing upon the MPTP metabolite MPP$^+$ to determine whether it or its production is necessary for the expression of neuronal toxicity.

In order to characterize the effects of MPTP in rat and guinea pig, pharmacological and behavioral effects have been studied in both species. In the rat, MPTP produced acute immobility, straub tail, retropulsion, but not analgesia or permanent motor impairment. There is a decrease in dopamine content in the substantia nigra in chronically treated rats, but no significant decrease in striatal dopamine. Intranigral administration of MPTP (10 ug daily for 5 days) failed to produce a 6-hydroxydopamine-like lesion.

The acute and chronic effects of MPTP on cultured bovine adrenal cells have been characterized as being those of a nicotinic agonist, whereas the p-Cl analog is a nicotinic antagonist. Exposure of cultured cells to MPTP for up to four days leads to changes in catecholamines, but not to the dramatic cell death seen in the primate nigrostriatal system.

The opiate properties of MPTP and 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP) have been assessed using in vitro receptor binding techniques as well as behavioral tests indicative of opiate action including analgesia, catatonia, respiratory depression and the loss of corneal and righting reflexes. All opiate activity was found to reside with MPPP, a potent u-type agonist, which was the initial synthetic product sought by illicit laboratories. The opiate properties of MPPP alone explain the repeated abuse of MPTP/MPPP mixtures by heroin addicts.

Longitudinal studies on urinary 6-hydroxymelatonin excretion as a measure of melatonin production by young girls during pubertal development have continued with urinary collections every six months. Urinary assays have thus far indicated a constant pattern of melatonin production through the early stages of pubertal development using Tanner criteria.

In instrumentation development, particle induced desorption has been used with the recently developed secondary emission mass spectrometer to determine the energy profile of organic ions emitted from surfaces. The energy differences between matrix and analyte ions can be used to an analytical advantage. Studies are in progress to utilize this experimental system as a molecular microprobe, to spatially locate and quantify organic compounds in a complex matrix such as tissue sections.

SECTION ON EXPERIMENTAL THERAPEUTICS
David C. Jimerson, M.D., Chief

Clinical research in the Section on Experimental Therapeutics focuses on neurochemical, neuroendocrine and pharmacological studies of major neuropsychiatric and psychosomatic syndromes in which disorders of neurotransmitter function are suspected. Over the past year clinical studies have focused on eating disorder syndromes including anorexia nervosa and normal weight bulimia The clinical design for studies of patients with anorexia incorporates psychobiologic tests when patients are at low weight, and at short and long-term intervals following weight recovery. Normal weight patients with bulimia are studied initially at a phase in which they have been involved with symptoms of bingeing and vomiting, and again several weeks later following abstinence from bingeing reinforced by the clinical program.

Study of neurotransmitter metabolites in cerebrospinal fluid (CSF) and plasma has continued. We have now compared metabolite levels for anorexic subjects with a history of weight loss bv fasting ("restricting anorectics" with levels for subjects with a history of bingeing and purging ("bulimic anorectics"). Serotonin turnover, as reflected in post-probenecid accumulation of the major metabolite 5-hydroxyindole acetic acid in CSF was significantly reduced in weight-recovered bulimic anorectics in comparison to restricting anorectics. Dopamine and norepinephrine metabolism showed no differences between the two patient groups. The difference in serotonin metabolism is consistent with differences in mood and impulse regulation in these patient groups. In patients with normal weight bulimia symptomatic phase, central dopamine turnover was reduced, with return to normal levels during abstinence from bingeing. Naturalistic study of bingeing and vomiting behavior in bulimic patients demonstrated progressive increase in the plasma ratio of tryptophan to other neutral amino acids. Further studies will attempt to clarify whether this change in plasma tryptophan alters brain tryptophan and serotonin levels.

Based on previous studies showing altered norepinephrine turnover in some phases of anorexia nervosa, we have extended this work to include assessment of noradrenergic receptor function using infusion of the alpha 2-adrenergic agonist clonidine. Underweight anorexic patients showed increased responsiveness to clonidine, while patients studied during the refeeding phase showed marked blunting of the growth hormone, norepinephrine, and 3-methoxy-4-hydroxyphenyl glycol (MHPG) responses to the drug. Thus, alterations in inhibitory pre-synaptic alpha-2-receptors may play a role in altered noradrenergic control of energy metabolism in anorexia nervosa.

Neuroendocrine studies in patients with eating disorders have focused on the hypothalamic-pituitary-adrenal and thyroid axes over the past year. In collaboration with Dr. P. Gold we have studied hormone responses to corticotropin releasing factor (CRF). Preliminary results in low weight anorexic patients have shown a submaximal ACTH response to CRF, but an exaggerated cortisol response. Studies of cortisol regulation in patients with normal weight bulimia showed normal serum and urinary free cortisol levels. In contrast, these patients manifested an abnormal high incidence of dexamethasone non-suppression when studied early after hospitalization and following an abstinence period of several weeks. Because of the role of thyroid hormones in modulating thermogenesis, we have assessed thyroid function during low weight episodes and following weight gain in patients with anorexia. The study of low weight patients showed reduced levels of thyroid hormones in comparison to control values, and submaximal, delayed thyroid stimulating hormone responses following infusion of thyrotropin releasing hormone.

As a continuation of previous studies of energy metabolism with eating disorders, we have assessed average caloric intake and found it to be reduced in patients with normal weight bulimia, in comparison to healthy control subjects. This result supports other evidence that these patients attempt to maintain body weight below a physiological set point. Plasma insulin levels were found to be low following the binge-free abstinent period in bulimic subjects, possibly as a consequence of long-term reductions in caloric intake. Our study of serum amylase in bulimic patients showed that blood levels of the enzyme are elevated following binge eating, and that serum amylase levels may be useful for monitoring compliance in treatment programs for bulimia.

Measurement of plasma norepinephrine metabolite levels in the evaluation of illness-related stress and sympathetic nervous system activation was assessed in several psychiatric syndromes. In collaboration with Dr. G. Ko, study of medication free schizophrenic patients revealed increased plasma MHPG levels with increased psychosis ratings in patients with chronic undifferentiated schizophrenia. This sympathetic response during increased psychosis may be syndrome specific, as paranoid schizophrenic patients did not show this relationship. In collaboration with Drs. L. Bierer and R. Post, the role of sympathetic nervous system activation in the hypercortisolemia of depression has been further investigated. Preliminary results of infusions with catecholamine receptor antagonists indicate that elevated cortisol levels in depressioon are not a direct result of the increased catecholamine release observed in some patients.

Further studies were conducted using the recently developed animal model of Parkinson's disease. The parkinsonism induced in human subjects exposed to the toxic drug N-methyl-4-phenyl-1-2,3,6-tetrahydropyridine (MPTP) is characterized by hypokinesia, rigidity, resting tremor, postural tremor, flexed posture, loss of postural reflexes, mutism and drooling. Thus, all the major clinical features of idiopathic Parkinson's disease are present. Study of subjects, exposed to MPTP through heroin abuse or laboratory contact revealed normal autonomic functions.

Study of the dopamine metabolite homovanillic acid in CSF revealed a 68% decrease in these subjects in comparison to controls. Treatment with L-DOPA resulted in a 65% reversal of the signs and symptoms of the syndrome. We have previously shown that systemic administration of MPTP in rhesus monkeys results in development of a neurological syndrome similar to parkinsonism. We have now demonstrated in brain tissue from treated monkeys a selective decrease in dopamine content in the caudate nucleus (12% of control values) and in the putamen (21% of control values). We have also shown that MPTP administration to beagle dogs produces similar behavioral and neurochemical deficits. In collaboration with Dr. L. Sokoloff, cerebral glucose utilization in MPTP treated animals is being evaluated using ^{14}C-2-deoxyglucose. As a whole, these studies continue to develop a model for studying the pathophysiology of, and treatment approaches to Parkinson's disease in man.

SECTION ON CHILD PSYCHIATRY

Judith Rapoport, MD., Chief

The Section conducts research on biological aspects of child psychiatry. Response to pharmacologic agents are current tools for this research, as well as newer neuroradiological techniques. Because research in child psychopathology is at an early stage of its development, a great deal of effort has gone into developing methodology for clinical research. This includes standardization of attentional tasks, ward ratings, and activity monitoring. For studies with preschool children, age appropriate structured situations are being devised to measure aggressive and hyperactive behavior. For learning disabled children, an epidemiological survey is being used to obtain truly representative learning disabled groups.

The response of normal and behaviorally disturbed children to dietary substances is being studied. Subjects are selected for alleged "sensitivity" to these substances. At present, glucose, sucrose and aspartame are being evaluated. These projects have in common the use of physiological responses correlated with behavioral change to understand the psychophysiology and pathophysiology in childhood behavior disturbance.

A large study has been completed in which the response to caffeine of grade school children who consume large quantitites of caffeine in their usual diet was compared to that of children with low caffeine consumption. The results showed that high consumers were different from low consumers even off caffeine (more motor restlessness and lower arousal levels) and had more "beneficial" and fewer negative side effects from caffeine. New studies will examine the response of children with anxiety disorders to caffeine in levels associated with current U.S. diet.

One major focus has been the hyperactive child, children with the diagnosis of Attention Deficit Disorder. A series of studies over the past seven years has addressed the question of a specific monoamine deficiency in this disorder. Results have supported multiple neurotransmitter system involvement rather than any single system.

Newer neuroradiological and electrophysiological brain imaging techniques are being used increasingly. Children with specific developmental disabilities are a particularly appropriate group for such studies, as their disabilities suggest specific neurological localization. A new series of studies has been initiated in which children and adults with specific developmental disabilities are examined using a variety of brain imaging techniques including: NMR, cortical evoked potential, and EEG tomographic mapping. For an adult dyslexic group, NMR, Xenon inhalation, CT scan and electrophysiological measures will be compared. The strength of these studies is that several imaging techniques are available at the National Institute of Mental Health, and can be compared in a single sample.

SECTION ON CLINICAL PSYCHOPHARMACOLOGY

William Z. Potter, M.D.

Our section was formed this year and includes three distinct components: a clinical research group with one senior and two junior psychiatrists working directly under the Chief; an analytical laboratory comprised of four technicians from our Institute and two from NIAAA which serves the needs of the intramural programs of both for catecholamine measurements in blood, cerebrospinal fluid and urine; and a basic pharmacology group under the leadership of Dr. Juan Saavedra.

In samples taken from patients under carefully controlled conditions, there are relatively high correlations between cerebrospinal fluid (CSF) and urinary MHPG, a major norepinephrine (NE) metabolite extensively studied in man. Correlations with plasma MHPG are less robust within the narrow range found in a more homogeneous population, suggesting that plasma MHPG is a rather insensitive measure of change. This is important given the number of studies here and elsewhere which depend on MHPG. Antidepressant treatments do produce reductions of MHPG but not as extensive as those observed in CSF or urine. Moreover, preliminary evidence reveals no discernible rhythm of plasma MHPG despite a well-documented pattern in urine. Factors other than output appear to be controlling plasma concentrations.

Studying the functional status of the NE system by comparing whole body turnover (the sum of NE plus its metabolites and urine), output of 6-hydroxymelatonin and plasma NE response to an orthostatic challenge provides much clear data on both pathophysiology and mode of action of treatment in depression. Tricyclics decrease total turnover, increase 6-hydroxymelatonin output and increase both absolute and relative NE responses to standing with an attendant increase in cardiovascular responses. A selective MAO Type A inhibitor, clorgyline, and ECT decrease turnover and selectively increase the relative NE increase on standing without altering cardiovascular parameters. The three treatments thus share the ability to increase the efficiency of the NE system despite other dissimilar effects. Concomitantly, we find that depressive illness is accompanied by dysregulation of the NE system characterized by the following inefficient responses: in bipolars supine NE tends to be low and shows a greatly exaggerated response on standing in the absence of increased cardiovascular responses: in unipolar depression supine NE is normal to slightly elevated (depending on antecedent stress) and is clearly elevated compared to controls on standing, again without increased cardiovascular parameters.

The analytical laboratory has measured thousands of samples during the last year from patients throughout the program. Plasma HVA is emerging both as a possible index of neuroleptic effect in schizophrenics despite previous negative studies as well as a marker of an apparent unsuspected circadian rhythm. Plasma NE and MHPG are routinely measured for numerous protocols with findings as summarized above. CSF measures of NE and DA as well as HVA, 5HIAA and MHPG are now routine. We are in the process of entering CSF data from almost 200 patients for systematic analysis to test several hypotheses concerning the distribution and interrelationships of these parameters.

Using CSF measures of the serotonin (5HT) metabolite, 5HIAA, and the NE metabolite, MHPG, we see significant decreases of both after three distinct antidepressants (desipramine, zimelidine and clorgyline) in man. A rat model for exploring functional interconnections between the NE and 5HT has been used in which a specific uptake inhibitor increases prolactin while NE inhibition does not, apparently the opposite of what has been found in man. Since most inferences concerning the role of 5HT in man are based on rat studies, these findings raise important questions and suggest that 5HT has a much greater role for neuroendocrine function in rat than in man.

Dr. Saavedra's group has developed methods for the quantitative analysis of receptor kinetics for neuropeptides in individual brain nuclei which allows us to study the regulation of the affinity and number of binding sites for neuropeptides in specific cell body groups and tracts within the central nervous system. These techniques open a new dimension in the study of brain neuropeptide systems.

We have recently found that brain angiotensin II binding sites are highly localized and differently regulated in specific brain areas. Kinetics of vasovasopressin, CRF, and somatostatin receptors can be similarly studied.

We propose to concentrate our efforts on the study of the regulation of neuropeptide receptors in specific brain areas related to the control of pituitary function. These experiments could help to understand some aspects of the neuropeptide contribution to the regulation of the response to stress and of their participation in psychosomatic diseases.

ANNUAL REPORT OF THE CLINICAL NEUROSCIENCE BRANCH
National Institute of Mental Health
October 1, 1983 - September 30, 1984

Steven M. Paul, M.D., Chief

Introduction

This is the second annual report of the Clinical Neuroscience Branch. The Branch consists of four sections, the Section on Preclinical Studies (Steven M. Paul, M.D., Chief), the Section on Molecular Pharmacology (Steven M. Paul, M.D., Acting Chief), the Section on Brain Biochemistry (Candace B. Pert, Ph.D., Chief), and the Section on Clinical Studies (David Pickar, M.D., Chief). The Branch continues to conduct a broad program of interdisciplinary neuroscience research. The current research interests of the various members of the Branch are therefore quite diverse, ranging from purely molecular studies of neurotransmitter/drug receptor structure and function, up to and including studies on psychiatric nosology. Although an attempt is made to focus attention on the major psychiatric disorders, and on the drugs and treatments that are useful in ameliorating such syndromes: individual investigators are encouraged to work on any problem(s) that is designed to unravel fundamental knowledge about the functioning of the nervous system. The major questions being addressed are essentially the same as those reported last year. New areas of investigation include several projects in the area of neuroimmunology initiated by Dr. Pert in the Section on Brain Biochemistry and Dr. Rick Weber in the Unit on Neuroimmunology. To date, Drs. Pert and Weber have examined the effects of a number of neuropeptides (and drugs) on the functioning of immune cells including lymphocytes and macrophages. This work, which will be reported on in somewhat more detail later, supports the notion that neuropeptides function in the immune network in a very similar fashion as they do in other neuroendocrine systems. In related studies, Dr. Birgit Zipser who has recently joined the Branch as a Guest Researcher during her sabbatical from Cold Spring Harbor will continue her work on the characterization of various monoclonal antibodies prepared against a simple invertebrate nervous system. Dr. Zipser will also be studying these antibodies using an autoradiographic technique developed in Dr. Pert's laboratory for visualizing antigen-antibody reactions in discrete regions of the rat brain. We are fortunate to have Dr. Zipser with us as she is one of the foremost authorities on the use of monoclonal antibodies to study neuronal circuits in the leech nervous system. Several other new personnel include a number of Guest Researchers and Visiting Fellows. Dr. Mary Lou Caspers has joined the Branch for one year during her sabbatical from the University of Detroit and is working on neuronal NA^+K^+ATPase and its possible regulation by various neurotransmitters. Dr. Yong Sik Kim has joined us as a Guest Researcher from the University of Seoul, Korea and will be working on a variety of projects including a further examination of changes in plasma homovanillic acid during treatment in schizophrenic patients, and the regulation of $GABA_\beta$ receptors by antidepressant drugs. Dr. Patrice Douillet has joined us on a training grant from M.R.I. (French Ministry of Research), and is working with Dr. Pickar on a careful and extensive statistical analysis of the diurnal alterations in plasma HVA in psychiatric patients and related clinical data from a variety of projects. Dr. Douillet has an expertise in biostatistics as it applies to psychiatric research and is specifically interested in examining issues of variability of biological measures over time. Dr. Rob Drugan has also recently joined the Branch as a Guest Researcher. Dr. Drugan has previously collaborated with Drs. Crawley and Paul on the application of an animal model of learned helplessness developed in Dr. Steven Maier's laboratory at the University of Colorado. These studies support a role of the GABA system in mediating the effects of aversive stimuli in various anxiety models and the relationship between inescapable "anxiety" or "fearfulness" and the subsequent development of learned helplessness (a so-called animal model of depression). Two additional Fellows who have recently joined the Branch, Drs. Mark Goldman and Dorota Majewska will be working in the Section on Molecular Pharmacology. Dr. Goldman will continue his studies on the isolation of an endogenous imipramine/serotonin uptake modulator from animal and human blood, and Dr. Majewska will be working on the regulation of benzodiazepine/GABA receptors by various peripheral steroids, including corticosterone. As is evident the Branch has experienced a slight growth spurt during the last six months due to the arrival of several Guest Researchers. It is gratifying that a number of scientists have been able to obtain their own funds to join the laboratory and work for a period of one to two years. All in all, this has been a rather busy year for the Branch as the changes that were initiated during our first year of operation have been completed are now coming to fruition.

SECTION ON PRECLINICAL STUDIES

Steven M. Paul, M.D., Chief

The members of the Section on Preclinical Studies continue to study the mechanism of action of important psychotropic drugs. The principal drugs under investigation include the benzodiazepines and other minor tranquilizers, tricyclic antidepressants, and the psychomotor stimulants, amphetamine and methylphenidate. Over the past year work on the benzodiazepine receptor has continued in a number of different areas. Primary emphasis have been given to the study of chloride transport. Dr. Rochelle Schwartz has demonstrated barbiturate/GABA receptor-mediated chloride transport in a novel subcellular preparation of brain. It is well known that the benzodiazepine/GABA receptor is an allosteric receptor complex which regulates the permeability of chloride ions through the chloride ionophore. A major problem in the field, however, has been the lack of a suitable *in vitro* method for measuring chloride transport-gated by the GABA receptor. Dr. Schwartz has succeeded in using a synaptoneurosomal preparation, which is subcellular brain preparation enriched in postsynaptic vesicles, to measure pharmacologically-relevant chloride transport. A number of papers are now in press concerning this method and a variety of experiments are underway to look at the effects of post-translational modifiers of the receptor ionophore complex. These studies are also an extension of Dr. Schwartz' previous studies using radiation inactivation to size the the various protein subunits of the benzodiazepine/GABA receptor complex. Our major working hypothesis at present is that the GABA receptor and other chloride ionophore-linked receptors (i.e. glycine) may share a common subunit, and it is our hope that we will be able to purify this subunit and gain insight into the molecular structure of these receptors. Dr. Dorota Majewska who has recently joined the laboratory has found that corticosterone appears to modify the benzodiazepine/GABA receptor complex suggesting an important feedback mechanism between the peripheral release of steroids during stress and their subsequent modulation of central GABA receptor "tone". These studies are now proceeding in an attempt to understand the mechanisms by which corticosterone modulates GABA receptor activity *in vitro* and to extend these observations to an *in vivo* preparation.

Other studies on the benzodiazepines involve the biochemical and pharmacological characterization of the so-called peripheral benzodiazepine receptor [using the specific ligand 4-chlorodiazepan (Ro 5-4864)]. We had previously demonstrated that Ro 5-4864 is a centrally active convulsant and that the convulsant properties of Ro 5-4864 are not mediated through the previously described peripheral benzodiazepine receptor, but rather appear to be mediated through the chloride ionophore itself. Electrophysiological studies carried out in the zona reticulata of the substantia nigra (Dr. Daniel Hommer) substantiate the potent excitatory actions of Ro 5-4864 and have demonstrated that such effects are selectively reversed by GABA receptor agonists such as the benzodiazepines or muscimol, but not by Ro 15-1788. Thus, Ro 5-4864-induced excitation is not mediated via the benzodiazepine recognition site itself. Studies by Drs. Rick Weber, Michael Ruff, and Candace Pert have characterized the peripheral benzodiazepine recognition sites on a variety of immune cells, and Drs. Ruff, Pert, Paul have demonstrated very potent effects of benzodiazepines in inducing chemotaxis in human macrophages. This effect is mediated by the peripheral benzodiazepine receptor since Ro 5-4864 is among the most potent drugs in inducing chemotaxis and its actions are blocked by PK-11195, a putative peripheral benzodiazepine receptor antagonist. These studies are among the first to conclusively demonstrate a pharmacological function for the peripheral benzodiazepine receptor and suggest that the sites located on other immune cells (e.g. lymphocytes), may play important roles in modulating immunological function. Other studies on the peripheral benzodiazepine receptor carried out in collaboration with Dr. Phil Skolnick have shown that the peripheral receptor has apparently developed rather late in evolution since a variety of invertebrate preparations and even lower vertebrates seem to lack peripheral receptors in the tissues that have been examined. In related studies, the pineal gland has been shown to have a high density of peripheral benzodiazepine receptors and these seem to be altered by changes in sympathetic outflow induced by either constant light, denervation, or the administration of various drugs. Thus, it appears that the peripheral benzodiazepine receptor may have different functions in different tissues. Drs. Mantione and Skolnick have also continued work on the isolation and purification of a substance present in high concentrations in antral stomach and brain that inhibits [^{3}H] Ro 5-4864 binding to the peripheral site and may represent an endocoid for the peripheral benzodiazepine receptor. These studies are also being carried out by Dr. Mark Goldman who has recently joined the Section on Molecular Pharmacology after spending a fellowship year in Dr. John Pisano's laboratory.

Previous studies in this Section have demonstrated the presence of high affinity of sodium-dependent recognition sites for [^{3}H] imipramine and [^{3}H] desipramine in brain. These sites have been shown to be structurally associated with presynaptic uptake or transport mechanisms for serotonin and norepinephrine respectively. Platelet membranes have also been used as an enriched tissue source for [^{3}H] impramine binding site and monoclonal antibodies have been raised against human platelet membranes. Dr. Itzchak Angel and Dr. Rick Weber are examining these monclonal antibodies and have shown that several seem to selectively inhibit serotonin transport in platelets and synaptosomes. The exact mechanisms responsible for this inhibition are now being studied and we are hopeful that these antibodies will give us information as to the function and regulation of the serotonin transport complex in brain in platelet membranes. More recently, studies on the dopamine uptake site labelled with [^3H] methylphenidate or a series of novel compounds represented by [^3H] GBR 12935 have been carried out. In addition to work attempting to characterize the [^3H] imipramine/serotonin transport complex in brain and platelet membranes, Drs. Angel and Goldman have continued their studies on the isolation of a blood factor(s) that preferentially inhibits the uptake of serotonin and the binding [^3H] imipramine to platelet and brain membranes. This factor in preliminary experiments may be altered by various endocrine manipulations and we are therefore attempting to quantify the material after such manipulations to see whether such changes may, in fact, be physiologically relevant.

Several investigators in the Section on Preclinical Studies including Dr. Itzchak Angel, MyDo Luu, Bridget Giblin, and Richard Hauger are pursuing studies on the regulation of a previously reported binding site for [^3H] (+) amphetamine in brain. Since our previous research indicated that these sites may be involved in the anorectic properties of phenylethylamine derivatives, we have examined the effects of food deprivation and refeeding on the density of these binding sites in various brain regions. Food deprivation of rats has been shown to result in a dramatic (30-50% reduction) in hypothalamic [^3H] amphetamine binding sites and brief periods of refeeding (2-4 hours), reverses this effect. These data suggest that the [^3H] amphetamine binding site may be involved in the regulation of feeding behavior. More recent studies by Drs. Angel and Hauger have shown that the changes in [^3H] amphetamine binding site density produced by food deprivation and refeeding are highly correlated to changes in blood glucose concentration. Moreover, in vitro studies have demonstrated that glucose, itself, can regulate the density of [^3H] amphetamine binding sites in hypothalamic slices. In the absence of glucose, incubation of hypothalamic slices in physiological buffers at 37°C results in a time-dependent decrease in [^3H] amphetamine binding, whereas the addition of glucose (1-30 mM) significantly stimulates binding in crude synaptosomal membranes prepared from the incubated slices. Moreover, the changes in [^3H] amphetamine binding induced by glucose in vitro are highly correlated to changes in [^3H] ouabain binding and the activity of Na^+K^+ATPase. These data suggest that amphetamine and related phenylethylamine derivatives may produce their anorectic effects by stimulating Na^+K^+ATPase activity in discrete hypothalamic nuclei involved in feeding and satiety. Currently, the effects of peripherally administered anorectic compounds, such as amphetamine and fenfluramine, on the effects on Na^+K^+ATPase activity are being examined using a recently developed autoradiographic technique.

Drs. Rodrigo Labarca and Aaron Janowsky have continued their work on the hydrolysis of phosphatydinositol (PI) (or the polyphosphoinositides) by various neurotransmitter agonists in brain slices. Since PI turnover is dramatically altered by many neurotransmitter agonists we have used the measurement of PI turnover as a measure of receptor activity. We, and others, have used lithium to amplify the effects of various neurotransmitter agonists in causing the accumulation of inositol-1-phosphate in vitro. Using brain slice preparations the effects of various neurotransmitters have been characterized and this system is currently being used for studies of the pharmacologic and physiologic regulation of these PI-coupled receptors. The effects of chemical and surgical lesions on neurotransmitter-mediated PI turnover in discrete in brain regions has also been examined. Drs. Labarca and Janowsky have observed that activation of protein kinase C by phorbol esters markedly inhibits the neurotransmitter-stimulated turnover of PI, indicating the possible existence of a negative feedback loop. More recently, Dr. Labarca has developed a method for studying PI turnover in a subcellular fraction of brain (the synaptoneurosome) and this should allow much more sophisticated experiments using various substances and compounds that are not capable of permeating brain slice preparations. The characterization of a suitable in vitro systems for studying PI turnover should prove useful in examining the regulation of the various receptors coupled PI turnover.

SECTION ON MOLECULAR PHARMACOLOGY

Steven M. Paul, M.D., Acting Chief

The Section on Molecular Pharmacology consists of two independent units, the Unit on Neurophysiology and Histochemistry (Drs. Lana Skirboll and Daniel Hommer) and the Unit on Behavioral Pharmacology (Dr. Jacqueline Crawley and John Glowa). Drs. Skirboll and Hommer have continued their studies in the characterization and functional significance of co-existing neurotransmitter systems in the central nervous system. Dr. Skirboll has continued her work utilizing immunocytochemical techniques combined with retrograde tracing with fluorescent dyes to extend her studies on various co-existing pathways in brain including those containing cholecystokinin (CCK), substance P, and serotonin. Moreover, with Dr. Eva Mezy and Jozcef Kiss, Dr. Skirboll has demonstrated the colocalization of CRH and CCK in specific hypothalamic nuclei. These studies have proven quite informative in unraveling the complexities of CRH release in the hypothalamus and thus the central regulation of the hypothalamic-pituitary adrenal axis.

Using extracellular single unit recording techniques, Drs. Hommer and Skirboll have continued their work on the interaction of CCK and dopamine in the substantia nigra. They have extended their initial finding on the effects of systemically adminstered CCK in producing dopamine autoreceptor supersensitivity. Iontophoretically applied CCK has been shown to potentiate the inhibitory action of iontophoretically applied dopamine. These findings have been interdigitated with those of Dr. Crawley (see below) on the effects of intracerebrally-administered CCK on dopamine-mediated behaviors. More recent studies have focused on the interaction of dopamine receptor antagonists and CCK electrophysiologic effects in the nigra. A series of studies are underway to see whether changes in dopamine autoreceptor sensitivity produced by CCK are shifted in animals who have been chronically treated with dopamine antagonists. Drs. Hommer, Skirboll, Palkovits, and Paul have recently completed a study demonstrating that selective vagal lesions (both peripheral and central) inhibit the effects of CCK on nigral excitation. However, none of these lesions altered CCK's ability to totally excite dopamine neurons suggesting that CCK may have both peripheral and central effects. In addition to examining the effects of CCK agonists on nigral dopamine neurons, Drs. Hommer and Skirboll have also examined the ability of two putative CCK antagonists to block the effects of systemically administered CCK. Proglamide and benzotript block the excitatory effects of CCK but at very large doses relative to previous reports. The potencies of proglamide and benzotript parallel their affinity for the brain CCK receptor, indicating that the electrophysiological techniques may provide a valid model for studying pharmacological actions of CCK antagonists as well as agonists.

Dr. Hommer has extended his studies of the electrophysiologic effects of the beta carboline derivative (βCCE) (which binds the benzodiazepine receptor as an inverse agonist) in various brain regions. The excitability observed in the substantia nigra zona reticulata following βCCE administration is reversed by the benzodiazepine antagonist, Ro 15-1788. Drs. Hommer and Skirboll have also observed that several different benzodiazepines potentiate the action of iontophoretically applied GABA in zona reticulata neurons and that the ability of these benzodiazpines seems to be positively correlated with their potency as hypnotics but not with their potency as anticonflict or anticonvulsant agents. In contrast to β-CCE's excitatory action in the zona reticulata of the substantia nigra the inverse agonist βCCE does not activate locus coeruleus neurons and thus it is unlikely that the latter is involved in the anxiogenic properties of various benzodiazpine receptor inverse agonists.

Dr. Jacqueline Crawley, Chief of the Unit on Behavioral Pharmacology has continued her studies in the behavioral action of neuropeptides. Dr. Crawley's major emphasis has been on studies of the neuromodulatory role of CCK on dopamine-mediated behaviors. Previous studies by Dr. Crawley have shown that CCK (in its sulfated form) directly applied to the nucleus accumbens markedly potentiates the dopamine mediated behaviors induced by either dopamine or apomorphine. The behavioral effects of CCK are antagonized by proglamide and by an antibody directed against CCK. Dr. Crawley has also demonstrated that peripherally administered CCK mimics a number of the behaviors associated with the "satiety state" and that these effects are completely abolished by peripheral vagotomy. Dr. Crawley has continued her interest in peptides as co-transmitters and their effects in potentiating or inhibiting the actions of the classical neurotransmitters including the biogenic amines and acetylcholine. She has developed several new approaches to understanding the possible physiological significance of co-existing neurotransmitters.

Drs. Crawley and Glowa have continued the Branch's studies on the behavioral pharmacology of "anxiety" using several novel benzodiazepine receptor antagonists. Dr. Crawley has characterized the behavioral syndrome produced by these compounds in rhesus monkeys. A number of putative anxiolytic substances have been examined for their abilities to reverse the βCCE-induced anxiety syndrome in monkeys, and interestingly several serotonin antagonists have been shown to be quite potent in blocking the behavioral and many of the physiological effects of βCCE. These results support the notion that serotonin is somehow involved in both the antianxiety effects of benzodiazepines and the anxiogenic effects of beta carboline inverse agonists such as βCCE. In related studies Drs. Crawley and Drugan have shown that the administration of FG 7142 mimics the development of learned helplessness in a behavioral paradigm developed by Dr. Steven Maier's group at the University of Colorado. These studies are among the first to integrate chemically-induced "anxiety" and behaviorally-induced "depression" and support clinical data that suggests a dimensional relationship between anxiety and depression in at least some depressed patients. Drs. Crawley, Drugan and Glowa continue their investigations on the anxiolytic properties of benzodiazepines in an attempt to dissect apart brain regions that are responsible for these effects. Attempts are underway to reverse the anxiolytic effects (as well as anxiogenic effects) of various benzodiazepine receptor ligands by central administration of selective antagonists. These studies may unravel a neuronal circuitry that mediates the behavioral effects of various anxiolytic and anxiogenic substances.

Dr. Marian Kafka has continued her studies on the characterization of alpha adrenergic and prostaglandin receptors in human blood elements. Specifically, she and her colleagues have measured changes in platelet α_2 adrenoreceptors in various neuropsychiatric disorders. She has extended her previous findings of alterations in platelet α_2 adrenergic receptor density in schizophrenia and depression and has worked out novel techniques for studying the coupling of receptors with various second messenger systems. More recently, Dr. Kafka has become involved in studies of polyphosphoinositide metabolism in brain particularly in the striatum. Dr. Kafka's work is attempting to define interactions between biogenic amine transmitters that are not normally considered to alter polyphosphoinositide metabolism and the classical muscarinic receptor that is coupled to PI turnover.

SECTION ON BRAIN BIOCHEMISTRY

Candace B. Pert, Ph.D., Chief

Dr. Candace Pert and her colleagues in the Section on Brain Biochemistry have extended their work on the characterization and visualization of neurotransmitter/drug receptors in the central nervous system. Using an autoradiographic method developed several years ago in collaboration with Dr. Miles Herkenham, in the Laboratory of Neurophysiology, Dr. Pert has characterized a number of pharmacologically relevant recognition sites for psychotropic drugs in the central nervous system (i.e., PCP, opiate receptor antagonists). In related experiments, Drs. Pert and Rothman have postulated the the presence of two types of opiate receptors, the delta receptor which appears conformationally fixed, and another opiate receptor which can assume the mu, delta, or kappa conformations. Dr. Pert and colleagues, including Drs. Paul Clarke, Rochelle Schwartz, and Agu Pert have mapped the nicotinic cholinergic receptor in brain using [^{3}H] agonists and the iodinated antagonist bungarotoxin. These studies clearly demonstrate that alpha bungarotoxin labels a distinct population of receptors that can be separated from the agonist-labelled nicotinic cholinergic receptor on the basis of their distribution in various brain regions. As mentioned above, members of the Section on Brain Biochemistry including Drs. Pert, Weber, and Ruff have been studying the modulation of immune function by various neuropeptides. Dr. Pert had previously shown that oat cell carcinoma of the lung is characterized by abherent fetal-like cells which secrete the brain neuropeptide bombesin. More recently she and Dr. Ruff have shown that these cells are indeed part of the immune system, since they have four surface antigens that have previously been shown to be specific to the human macrophage. In related experiments, human macrophages have been shown to chemotax towards opiates, including opiate agonists such as morphine and enkephalin, and these effects appear to be mediated via an opiate receptor, since they are reversed by naloxone in stereoselective fashion. Other studies being carried out are designed to characterize the effects of various endorphins on the immune network. Drs. Weber and Pert are examining the effects of neuropeptides on a number of *in vitro* measures of lymphocyte function. In addition to studying the effects of various neuropeptides and neuromodulators on immune function, Dr. Pert and her colleagues have set up a novel immunohistochemical assay for visualizing antibody patterns in brain using neuroanatomically preserved frozen sections. This technique has been applied to visualizing and localizing the transferon receptor in brain as well as the distribution of a number of opiate peptides. Drs. Pert, Weber, and Knight have been examining the possibility that schizophrenia has an autoimmune component. Although previously postulated by many investigators no conclusive evidence exists to suggest that autoantibodies are secreted in schizophrenic patients. Drs. Pert and Knight have set up some very sensitive method for detecting autoantibodies to specific brain antigens and are in the process of screening sera from schizophrenic patients to see whether significant antiantibody reactions can be detected.

In a collaborative effort with Drs. Pert, Ken Rice, and Steven Larson a newly synthesized opiate antagonist has been shown to be useful as a positron emitting isotope for PETT studies in living animals. A fluorinated (F-18) derivative of this compound ^{18}F-3-acetylcyclofoxy has been synthesized and used to probe the opiate receptor in monkeys. These studies are quite encouraging and suggest that this compound will be useful for clinical studies in man.

SECTION ON CLINICAL STUDIES

David Pickar, M.D., Chief

The research program of the Section on Clinical Studies involves systematic biological and pharmacological studies of schizophrenia and depression and of related clinical studies of the endogenous opioid and benzodiazepine systems in man. The clinical research for this program is based on the 4-East Nursing Unit of the NIH Clinical Center and and many studies involve collaborations with investigators outside of the Section.

A major focus of the Section's studies on schizophrenia relates to the mechanism of action of antipsychotic medication. Previous results have shown that treatment with the neuroleptic, fluphenazine, is associated with a time-dependent decrease in plasma levels of homovanillic acid (HVA). This time-dependent decrease in plasma HVA is consistent with preclinical data suggesting that chronic neuroleptic treatment decrease dopamine neuronal activity in dopamine-containing neurons in the mesencephalon. We have also observed that this decrement in plasma HVA is correlated with clinical response, a finding which suggests that the mechanisms of neuroleptic action may more closely be associated with alterations in presynaptic activity than postsynaptic blockade. Ongoing studies are in progress to further elucidate the mechanism of action of neuroleptic medication and to evaluate whether measurement of plasma HVA represents an accurate reflection of central dopaminergic activity. A double-blind crossover treatment trial with the calcium channel blocker, verapamil, and the neuroleptic, fluphenazine, is currently underway and a comparative study which may aid in examining pharmacologic approaches to alter dopaminergic functioning.

Our work in depression was initiated as a project headed by Dr. Alec Roy who has systemically examined the purported biological tests for depression. The success of recruitment of patients for this study has lead to a broader program investigating the neurobiology of depressive illness. Patients with DSM-III diagnosed melancholia in a present or previous depressive episode have higher circulating levels of plasma norepinephrine (NE) than do controls or nonmelancholic depressed patients. Further, melancholic patients who are nonsuppressors on the dexamethasone suppression test (DST) were found to have the highest levels of plasma NE suggesting an association between activation of the sympathetic nervous system and the HPA axis. In studies using cerebrospinal fluid (CSF) methodologies, decreased levels of dopamine metabolites in melancholic patients when compared with nonmelancholic depressives have been observed and DST nonsuppressors have higher levels of CSF MHPG than do suppressors. The notion of diminished CNS dopaminergic functioning in depressive illness is currently being investigated by studying plasma HVA levels prior to and during pharmacotherapy. Urinary catecholamine metabolites are also being measured to further investigate possible disturbances in noradrenergic and dopaminergic function in depression. In collaboration with Walter Lovenburg, Ph.D., and Seymour Kaufman, Ph.D., a pilot investigation of the therapeutic effects of tetrahydrobiopterin (BH_4), a pterin cofactor required for catecholamine synthesis has been carried out. Although no therapeutic benefit was observed in several depressed patients treated with BH_4 (1 mg/day for one week, 2 mg/day for two weeks) an increase in plasma HVA and MHPG during BH_4 treatment was documented. BH_4 is still being evaluated for possible antidepressant effects.

The Section has also continued its work studying the endogenous opioid system in man. Dr. Martin Cohen who left the Section during this past year had initiated a series of studies using high doses of naloxone to study behavioral and physiological implications of the endogenous opioid system. The finding of dose-dependent behavioral and endocrine effects of high-dose naloxone have suggested a tonic role for the endogenous opioid system in behavior and in selected physiological variables. Previous work demonstrating a stress response role for the endogenous opioid system has been followed by the intrathecal administration of synthetic β-endorphin into patients with disseminated malignancy. The observation of a confusional hypomania in one patient and some antidepressant effects in the other suggest that this endogenous peptide is behaviorally active when administered at site with access to the CNS. We have continued to utilize naloxone administration strategies with particular emphasis on studying effects on eating behavior in patients with exogenous obesity and as a tool for studying HPA axis activity in psychiatric patients. These two latter studies are currently in progress.

Dr. Daniel Hommer has initiated a new area of research in the Section. This research involves using diazepam infusions to measure benzodiazepine receptor sensitivity in humans. Dose response curves are constructed using saccadic eye velocity and selected endocrine and behavioral responses are used as a reflection of benzodiazepine receptor sensitivity. This work is intended to begin systematic investigation of the benzodiazepine system in man, and will in the future, (Ro 15-1788) utilize the administration of the newly developed benzodiazepine antagonist to psychiatric patients and normal volunteers.

Dr. Rex Cowdry has directed an outpatient program studying borderline illness and related dyscontrol behavior. The completion of a crossover trial of a benzodiazepine, phenothiazine, and monoamine oxidase inhibitor represents a landmark study which provides new and important data regarding treatment of this very difficult illness. Continuing work using EEG techniques and procaine infusions may help in gaining an understanding of the biological substrates of the borderline syndrome.

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National Institute of Mental Health

Mortimer Mishkin, Ph.D., Chief

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Two Visual Pathways

The striate cortex in the monkey is the source of two functionally dissociable visual pathways. One courses ventrally interconnecting striate, prestriate, and inferior temporal cortex and is crucial for the visual identification of objects; links between this occipitotemporal pathway and limbic structures in the temporal lobe as well as ventral portions of the frontal lobe appear to make possible the cognitive association of visual objects with other events, such as emotions and motor acts. The other visual pathway runs dorsally, interconnecting the striate, prestriate, and inferior parietal cortex and is critical instead for the visual localization of objects; links between this occipitoparietal pathway and both dorsal limbic and dorsal frontal cortex enable the cognitive construction of spatial maps, as well as the visual guidance of motor acts that may have been triggered initially by activity in the ventral pathway. In contrast to the occipitotemporal pathway, which remains modality specific throughout its course, the later stations in the occipitoparietal pathway receive convergent input from other modalities and so constitute polysensory areas. We have demonstrated this entire visual system at work through application of the [^{14}C]2-deoxyglucose method. By comparing a visually deafferented and a normal hemisphere in the same monkey, we found that all tissue related to vision can be clearly identified on the basis of differential metabolic activity in the two hemispheres during visual stimulation. The studies were carried out in awake monkeys that either were passively exposed to visual patterns in a rotating drum or actively performed a pattern discrimination for water reward. In both situations reduced glucose utilization in the deafferented as compared with the normal hemisphere was found cortically throughout the entire expanse of the two visual pathways described above. The comparison revealed visual-nonvisual borders that were sharp and highly consistent among animals, allowing delineation of the exact limits of visually related tissue. These limits were found to enclose a larger cortical territory than is generally recognized as being related to vision, including the fundus and part of the upper bank of the intraparietal sulcus, the posterior part of the parahippocampal gyrus, and the lateral bank of the rhinal sulcus. The borders appeared reliably at zones of architectonic transition, lending new functional validity to cortical architectonics. Also, tissue related to vision could be followed subcortically into parts of the limbic and striatal systems.

Anatomical organization of prestriate cortex

To trace in detail the flow of visual information through the large expanse of visual cortex revealed by the metabolic studies, we have undertaken a series of anatomical studies using axonal transport and degeneration techniques. The goal is to identify the multiple visual areas within the prestriate cortex, explore their organization, and map their projections forward into both the temporal and parietal lobes. In the course of these studies, we have developed a myeloarchitectural stain that clearly distinguishes among the multiple prestriate areas for the first time.

Our findings confirm the conclusion that the striate cortex is the source of two major cortical projection systems. The first system begins with the striate projection to the second visual area, V2, which in turn projects to areas V3 and V4. These three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like striate cortex, each belt contains a complete representation of the visual field. Area V2 corresponds to Bonin and Bailey's prestriate area OB, while V3 and V4 are both contained within their prestriate area OA, exclusive of its dorsal part. Area V4 in turn projects to areas TEO and TE in the inferior temporal cortex.

The second major system begins with both striate and V2 projections to visual area MT, which is located in the caudal portion of the superior temporal sulcus, mainly within dorsolateral OA. Area MT in turn projects to four additional areas in the upper superior temporal and intraparietal sulci. Although the total extents of these four areas are not yet completely established, the more anterior one in the intraparietal sulcus clearly falls within Bonin and Bailey's inferior parietal area PG. Thus, one major system of projections out of striate cortex is directed ventrally into the temporal lobe, while a second is directed dorsally into the parietal lobe. Furthermore, the divergence between these two systems appears to begin almost immediately after striate cortex, i.e. in its initial projections. While continuing to map these two corticocortical systems, we have also begun to investigate their subcortical connections. Our findings indicate that, like striate cortex, both areas MT and V4 project to the inferior and lateral pulvinar, superior colliculus, and reticular nucleus of the thalamus, but unlike striate, both prestriate areas project in addition to the putamen, caudate, claustrum, and pontine grey. The considerable overlap in the subcortical projections from MT and V4 suggest that the contribution of each area to subcortical processing lies not in a unique set of subcortical projections but rather in the unique information each supplies.

Prestriate cortex and the analysis of motion and figure-ground relationships

Now that we know the location, topography, and connections of many of the prestriate subdivisions, we can compare their neuronal properties and trace the transformation of visual information through them. We are particularly interested in visual areas MT and V4, as our anatomical experiments have shown that each is a critical part of a different pathway. Indeed, our neurophysiological evidence indicates a striking functional specialization of the two pathways. In area MT, which is known to be insensitive to either the form or color of a stimulus but highly sensitive to direction of stimulus motion, we have discovered an elaborate columnar system for direction of motion, comparable to the columnar system for orientation discovered by Hubel and Wiesel in striate cortex. Within a vertical column in MT, all cells respond to the same direction of motion. Moving across the columns, the preferred direction of motion changes systematically, resulting in a clock-like representation of direction. The sensitivity of MT neurons to where a stimulus is moving, rather than to what the stimulus is, is consistent with the strong anatomical connections of MT with areas of the posterior parietal cortex, which is known from our neurobehavioral studies to be necessary for processing the spatial relationships among objects.

In contrast to neurons in MT, those in V4 are insensitive to motion but are highly sensitive to form and color. As in striate cortex, some neurons in V4 are sensitive to object contours and low spatial frequencies while others are more sensitive to textures and high spatial frequencies. Thus, the data on V4 suggest that within each visual area of the occipitotemporal pathway, the contours of object surfaces and the textures of object surfaces may be processed by separate populations of neurons. Surface color also appears to be processed by neurons within each of these visual areas. The results argue against the prevalent view that each area of the occipitotemporal pathway processes a different aspect of an object separately, such as color in V4 and depth in V2, and suggest instead that the different features of an object are processed in parallel within each area. At the same time, the results are consistent with our anatomical evidence indicating that the occipitotemporal pathway is organized as a serial hierarchy.

A hierarchical model predicts that the product of visual processing will become progressively more complex at each successive stage in the pathway. So far, our results in V4 support this prediction. In addition to sensitivity to the contour, texture, and color of a stimulus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. This responsivity to stimulus differences in the receptive field is due to a unique receptive-field structure of the neurons in V4: a small excitatory receptive field surrounded by a large, silent, suppressive zone. The surround zone is silent in that stimulation of it alone is without effect, but it is also suppressive in that it has properties antagonistic to those of the excitatory field and, hence, can suppress the response to an excitatory-field stimulus if the surround is stimulated in the same way. Thus, V4 neurons may play a role in separating figure from ground, a fundamental requirement for object perception. Like the properties of MT, those of V4 are consistent with its anatomical connections, since V4 is a crucial link in the relay of object-quality information to inferior temporal cortex.

Inferior temporal cortex and the analysis of shape

The discrimination of complex visual stimuli normally depends on a variety of features, such as brightness, size, texture, and color, but probably the most salient visual feature is shape. In previous studies of the properties of inferior temporal neurons, we found that most neurons were in fact highly sensitive to the shapes of objects. Since very few of these neurons are sensitive to an object's local features, such as the location and orientation of single edges, they presumably code some global measure of shape. The problem is to identify that measure.

It is well known from the computer pattern-recognition literature that a particularly potent global measure of shape is the Fourier descriptor. The Fourier descriptors are a basis set of shape functions that can be used to generate any shape; likewise, any object can be decomposed into a unique set of these shape descriptors. The descriptors thus provide a powerful method for classifying shapes. Is it possible that inferior temporal neurons use such a method? To answer this question, we have been studying responses of inferior temporal neurons to a set of Fourier descriptors. So far, over half the neurons tested have been sharply tuned to the set, that is, each of the tuned neurons responds best to a particular descriptor in the set. Furthermore, most of the tuned neurons exhibit size constancy in that they respond best to a particular descriptor regardless of size. These results strongly support a Fourier descriptor-like model of shape analysis. We are continuing to investigate other models of shape analysis, however, especially spatial frequency decomposition, to determine if the Fourier descriptor model is in fact the best explanation for our results.

Additional indication of the importance of inferior temporal neurons for shape or pattern analysis comes from studies on the influence of selective attention on the response of these neurons. The studies have shown that when a monkey is required to shift its attention but not its fixation in order to detect the dimming of a small bar of light presented outside the fovea, the discharge of most inferior temporal neurons to the appearance of the bar is decreased below that seen when no attention to it is required. However, when the attentional demand is changed so that the monkey must determine whether the stimulus presented is the bar or some other pattern, then the neuronal discharge to the same bar is often enhanced. The results suggest the interesting possibility that, in inferior temporal cortex, attention to the pattern of a stimulus increases neuronal activity, whereas attention to the spatial location of the same stimulus, which may be all that is required to detect its dimming, suppresses activity; the latter result could be due to a suppressive influence from the dorsal cortical visual pathway, which is specialized for spatial vision.

The pattern of a stimulus is not the only attentional target that can elicit enhanced responses in inferior temporal neurons. The evidence comes from a discrimination task involving four stimuli composed of all combinations of two outline shapes, square and circle, and two internal textures, stripes and dots. Either stimulus dimension may be made relevant, e.g. the reward may be made contingent on immediate response to onset of a square and delayed response to onset of a circle; or the same can be done with stripes and dots; or, indeed, any other arbitrary rule can be adopted. Preliminary results indicate that an enhanced neuronal response may be elicited by attention to texture as well as to pattern. Furthermore, some neurons may show enhanced activity when an immediate response to a stimulus compound is required as compared to the activity that is evoked when there is a delayed response to that same compound. For other neurons, a change in response to a stimulus may occur when the old rule governing the discrimination has been changed and only while the monkey is discovering the new rule.

Changes in neuronal response with shifts in attention are not always reflected simply in changes in the strength of neuronal discharge. In some cases, a neuron may show a sustained response when one rule is in effect and a transient response when another rule governs. Other discharge characteristics that may change are response latency and consistency of response onset. These observations suggest that single neurons might be using a temporal code to signal information about the visual stimulus. To investigate this possibility, we have developed a new technique which involves repeated presentation of a large set of mathematically related stimulus patterns while the responses of a single neuron are being recorded. The responses of the neuron to the entire set are then transformed into a continuous probability density function and the response to each stimulus decomposed into its principal components, or waveforms. Statistical analysis has shown that these waveforms, which represent the temporal discharge pattern and not just the mean discharge rate of the neuron, are differentially associated with the stimulus patterns. The first three or four of the principal components are usually adequate to represent the neuronal response to each pattern, and these first three or four waveforms are remarkably similar from one neuron to another not only in inferior temporal cortex but also in striate cortex. The results suggest that there is an underlying temporal code made up of a small number of spike sequences that neurons may use to convey information about complex visual patterns from one station to the next along the stimulus processing chain.

Inferior temporal cortex and stimulus equivalence

Stimulus equivalence across retinal translation is the phenomenon whereby a stimulus is recognized as the same regardless of its location in the retinal field, even if the different loci project to different hemispheres. Since nearly two-thirds of inferior temporal neurons receive visual information not only from their own hemisphere but also from the opposite hemisphere via the forebrain commissures, we have proposed that these neurons provide the mechanism for such equivalence. To test this hypothesis, we prepared monkeys with bilateral inferior temporal ablations combined with section of the optic chiasm and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control monkeys (with either inferior temporal lesions alone or chiasm-section alone), who can perform immediately with the second eye any discrimination that they have learned with the first, the experimental monkeys had to learn each discrimination anew with the second eye. These results support the proposal that stimulus equivalence across the visual hemifields depends on the convergence of their projections onto single inferior temporal neurons. Since inferior temporal neurons have not only bilateral but also large receptive fields, they could of course be responsible for stimulus equivalence across any retinal translation, within as well as between the hemifields. We are currently testing this more general version of the hypothesis by training monkeys to discriminate a pair of patterns in one quadrant of a visual hemifield and then testing them for transfer to the other quadrant. If our hypothesis is correct, transfer should be impaired in this situation following inferior temporal lesions alone.

In addition to the inferior temporal area TE, the border of areas OC and OB, selected parts of area OA, and extensive parts of area TEO are also reciprocally connected through the forebrain commissures. Since the interhemispheric transfer of visual information is critically dependent on these commissures, we have attempted to localize and quantify their contribution to vision by application of the 2-deoxyglucose method. For that purpose, we measured rates of glucose utilization throughout the ventral cortical visual system in two different surgical preparations, one with unilateral optic tract section combined with forebrain commissurotomy, and the other with unilateral optic tract section alone. The commissural contributions to vision were inferred from differences in metabolic activity between the visually deafferented hemispheres of the two groups.

Results indicated that there were no differences between groups in any area from the OC-OB border through area TEO, where glucose utilization in the deafferented hemisphere averaged 60% of that in the intact hemisphere. For area TE, however, metabolism in the deafferented hemisphere of animals whose commissures were cut remained at 60% of that in the intact hemisphere, whereas in animals whose commissures were preserved the value rose to 80-90% of that in the intact hemisphere. The results provide clear evidence of the commisural input to area TE but, surprisingly, not to any part of the visual system posterior to it.

On the possibility that commissural activation of the prestriate-posterior temporal region in a hemisphere deprived of a direct visual input might be evident only against a background of spontaneous retinal discharge, we prepared a third group of monkeys with midline section of the optic chiasm combined with occlusion of the right eye. Despite the preservation of the retino-geniculo-cortical projection in this preparation, the results still failed to reveal a commissural contribution to the prestriate-posterior temporal zone.

A likely solution to this puzzle has come from our recent studies of neurons in area V4, the largest visual area in cytoarchitectonic area OA. As indicated earlier, the receptive fields of V4 neurons have large, silent, suppressive surrounds. That is, a stimulus placed in the surround of the receptive field, though without effect itself, can nonetheless suppress the response to a stimulus placed within the receptive field (a proposed mechanism for figure-ground separation). Recently, we discovered that the suppressive surrounds of V4 neurons extend far across the vertical midline into the opposite visual hemifield and that this suppression is eliminated by section of the corpus callosum. Thus, the commissural inputs to V4 appear to be largely suppressive, in contrast to the commissural inputs to area TE, which are known to be excitatory. If we were to find that neurons in the rest of the prestriate zone also receive suppressive rather than excitatory inputs from the opposite hemisphere, we will not only have resolved the paradox presented by the 2-deoxyglucose data but will also have provided an explanation for another previously puzzling fact, namely, that despite heavy interconnections across the callosum, prestriate neurons, unlike neurons in area TE, have only contralateral excitatory fields.

Inferior temporal cortex and recognition memory

The spatial convergence on single cells in area TE provides a mechanism that is essential for discrimination learning under normal viewing conditions. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite fluctuations in fixation, distance, direction, and angle of regard, fluctuations which necessarily lead to activation of different populations of striate and prestriate cells on the successive presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the effects of experience or training. We now have evidence that the inferior temporal cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Trained monkeys that are shown an object once will demonstrate that they recognize it as familiar several minutes later by avoiding it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus leaves a trace against which a subsequent presentation of that same stimulus can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity. The area in which the neural trace is first established appears to be area TE, since removals here but not elsewhere in the visual system abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, area TE contains the traces laid down by previous viewing, and these serve as stored representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay or be renewed or even refined, while new traces are added to the store. To study these postulated visual traces at the single-unit level, we are investigating whether a neuron that at first responds equally to two similar stimuli can be induced to respond differently to them by training the animal to attend to their physical differences.

A limbo-thalamic circuit and recognition memory

In the process of investigating the role of other temporal-lobe structures in object recognition, we obtained a result that is particularly exciting because it may help to solve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. We have found in our studies, however, that if damage to the hippocampus is combined with damage to the amygdala then a profound recognition loss ensues, a loss that is even greater than that produced by lesions of inferior temporal cortex.

Before the implications of this finding are considered, it should be noted that there was an alternative explanation for the severe effects of the combined lesion that needed investigation. That is, neither the amygdalectomy nor hippocampectomy had included all of the entorhinal cortex, whereas the combined lesion did. The results were therefore consistent with the possibility that the severe recognition loss after the combined lesion was actually due to destruction of the entorhinal cortex. A test of this possibility has now shown that even when total entorhinal ablation is combined with hippocampectomy the recognition impairment is no greater than after hippocampectomy alone, whereas when total entorhinal ablation is combined with amygdalectomy the impairment is just as severe as it is after combined removal of the amygdala and hippocampus. In short, the entorhinal lesion appears to be equivalent to a hippocampectomy in this situation, presumably because it disconnects the hippocampus from the inferior temporal visual cortex. The results thus support our earlier conclusion that combined damage to the amygdaloid and hippocampal systems is necessary to produce a profound recognition impairment in monkeys. That discovery has not only opened up a new possibility for resolving the discrepancy between clinical and animal findings but has also led to new insights into the neural mechanisms of memory.

According to our present model, object recognition depends on a cortico-limbo-thalamic pathway, activation of which lead to the storage in cortex of the trace or representation of the stimulus that gave rise to the activation. In the case of vision, as already indicated, the cortical tissue in which the storage is presumed to take place is area TE. However, as will be described in a later section of this report, other sensory modalities appear to be organized neurally along lines similar to those of the visual modality, and each is presumed to be represented by a final cortical processing station located in the anterior temporo-insular region with functions analogous to those postulated for area TE. We know that each of these final stations in the sensory system of the various modalities projects both to the amygdala and, via relays in the perirhinal and entorhinal cortex, to the hippocampus. Furthermore, each of the limbic structures projects to the adjacent parts of the medial thalamus, the amygdala via the amygdalofugal pathways to the magnocellular portion of n. medialis dorsalis (MDmc), and the hippocampus via the fornix to the anterior nuclei (Ant N). Finally, we have shown that, in vision, one-trial object recognition is severely impaired not only by area TE lesions, or combined amygdalo-hippocampal lesions, but also by disconnection of area TE from the amygdalo-hippocampal complex, disconnection of the amygdalo-hippocampal complex from their thalamic targets, or combined destruction of these thalamic targets.

Since the limbo-thalamic pathways are so critical for recognition memory, we have been investigating whether their prefrontal projections might also be involved in this function. So far we have determined that removal of ventromedial prefrontal cortex (the target of both MDmc and Ant N) yields a severe recognition loss, whereas removal of either dorsolateral or inferior prefrontal cortex (both targets of the parvocellular division of MD) produces little impairment. The discovery of a possible functional relationship between the ventromedial prefrontal cortex and the limbic system provides an important new clue to the organization of prefrontal cortex. That is, for certain memory functions, the dorsolateral prefrontal cortex may depend on interaction with the ventromedial prefrontal region in much the same way that anterior temporo-insular cortex depends on interaction with the medial temporal region. We are currently testing whether, within the ventromedial prefrontal region, the orbital cortex (to which MDmc projects) and the adjacent anterior cingulate cortex (to which Ant N projects) contribute equally to recognition memory, just as we had found to be the case for all the earlier segments of the amygdaloid and hippocampal systems.

Our experimental evidence that combined damage to these two limbo-diencephalic systems is necessary to produce a disorder in monkeys resembling the syndrome of global amnesia in man is consistent with most of the neuropathological evidence available on amnesic patients, including patients with temporal lobe resections and those with Korsakoff's Disease. One piece of clinical evidence does not seem to fit this view, however, and supports instead the view that damage to the hippocampal formation alone is sufficient to produce the syndrome. The evidence comes from amnesic patients with diseases of the posterior cerebral artery, which is known to provide the blood supply of the hippocampus but not the amygdala. From the few clinical studies that included neuropathological reports, however, it appears that although the infarction always includes the posterior part of the hippocampal formation, it may also invade the inferior temporal cortex, stria terminalis, thalamus, or combinations of these, raising the possibility that hippocampal damage alone is not responsible for the memory loss. To examine this issue experimentally, we have initiated a study on the effects of occluding the posterior cerebral artery in the monkey. Our preliminary findings indicate that such occlusion will produce substantial visual recognition loss in some monkeys but not all. Correlation of these findings with the neuropathology may thus resolve the question regarding the locus of damage responsible for the amnesia in patients with posterior-cerebral-artery disease.

The cholinergic system and recogntion memory

In the neural model that we have proposed to account for recognition memory, a stimulus leaves a lasting trace in the sensory modality's highest order processing station whenever that stimulus activates the cortico-limbo-thalamic pathway described above. This hypothetical circuit is incomplete, however, because it does not specify the link through which the limbic and thalamic structures reactivate the sensory cortical areas that activated them. One possible candidate for this missing link is the basal forebrain cholinergic system. Two major lines of evidence support this view. First, cholinergic agonists and antagonists have long been know to influence many forms of memory in many species, and in a recently completed study of our own we found that the same holds true for recognition memory in the monkey. Second, recent neuropathological studies in patients with Alzheimer's disease, who often show a marked memory loss as one of their earliest symptoms, revealed selected cell loss in both the nucleus basalis of Meynert (nbM), the major source of cholinergic input to the cerebral cortex and amygdala, and the nuclei of the diagonal band of Broca and medial septum (dbB+ms), the major sources of cholinergic input to the hippocampus. This cell loss is accompanied by markedly decreased cortical and limbic levels of choline acetyltransferase and acetylcholinesterase, enzymes involved in the synthesis and metabolism of acetylcholine.

In recent experiments performed in collaboration with investigators from The Johns Hopkins Medical School, we have produced a recogniton memory impairment in monkeys by damaging these basal forebrain areas with a neurotoxin. Histological and neurochemical analyses, completed on some animals only, indicate that the animal with the most severe memory impairment had nearly complete destruction of the basal forebrain system as well as a 60-90% loss of choline acetyltranferase (ChAT) activity across most of the cortex. However, there was much less loss of ChAT activity in area TE, the cortical sector that is so critical for visual recognition memory. The results suggest the interesting possibility that the visual recognition impairment in the animals with the combined lesions resulted from cholinergic denervation not of the cortex but of the limbic system. This possibility would also account for the finding that only combined damage of nbM and dbB+ms yielded impairment, since only such combined damage would result in cholinergic denervation of both the amygdala and hippocampus.

In conjunction with these experimental manipulations of the basal forebrain cholinergic system, the goal of which is a better understanding of the nature and neurobiological basis of the cognitive losses associated with Alzheimer's Disease, we have begun to examine the cognitive losses associated with normal aging in the monkey. Like the neurotoxic-lesion study, this one also is being performed in collaboration with investigators from The Johns Hopkins Medical School. Our initial results have demonstrated a moderate but systematic decline in the recognition memory of monkeys from early adulthood (3-6 yrs), through middle age (14-17 yrs), to old age (17-30 yrs). Since we know much of the neuroanatomical substrate for this form of memory, we can now examine that substrate for any evidence of increasing pathology with age.

Amygdala, hippocampus, and associative memory

According to our neural model, once the trace or representation of a stimulus has been stored in the highest-order processing station for any given modality, that stored trace can enter into association with the stored traces of other stimuli and other events, thereby providing the stimulus with associative meaning. As has been indicated, the amygdala and hippocampus, as well as their separate efferent pathways and separate thalamic targets, make approximately equal contributions to recognition memory, presumably reflecting their roughly equal contribution to the cortical storage of stimulus traces. In the case of associative memory, however, our results indicate that the amygdala and hippocampus make very different contributions.

In one experiment, monkeys were trained preoperatively on a visual recogntion task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two objects visually after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, but the amygdalectomized monkeys fell to chance performance.

Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this task, the animal was required to remember on the test trial where on a three-well tray each of two different objects had been presented on the acquisition trial. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance.

The results of these two complementary experiments indicate that, although both the amygdala and hippocampus are critical for certain forms of associative memory, their roles are totally different. Many further analyses along the lines of these experiments will of course be necessary before the selective associative memory functions of the amygdala and hippocampus can be identified with confidence. For example, the association of an object with an affective state, such as fear or pleasure, appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view has been obtained in an experiment showing that one-trial object-reward association is impaired more by amygdaloid than by hippocampal lesions, although neither deficit approaches in severity the one produced by combined removal of these two structures. By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

A cortico-limbic pathway in somesthesis

The work described above elucidating a cortico-limbic system for visual perception and memory has led to the search for analogous systems in other modalities. So far, analogous anatomical pathways have been found for both taste and audition, but because of a recent finding that combined amygdalo-hippocampal lesions produce severe recognition loss not only in vision but also in somesthesis, particular attention has been paid to the pathway in this modality. Also, anatomical studies on the somatosensory system have been supplemented by electrophysiological studies in order to permit functional comparisons with the cortico-limbic system in vision.

Injections of cell markers were made into the hand representation of physiologically identified cortical fields lying in or near the lateral sulcus of the monkey and the results compared with earlier findings from injections in postcentral cortex. The laminar pattern of termination of the projections of the various cortical fields suggests the sequential order in which they process somatosensory information. In the visual system, forward corticocortical projections (i.e. outward from striate cortex) are characterized by a heavy input to layer IV, whereas backward projections (i.e. towards the striate cortex) completely avoid layer IV and instead have a characteristically heavy input to layer I. By analogy with these findings in the visual system, our results in the somatosensory system indicate that the forward sequence of projections is: primary sensory area (SI) to the secondary sensory area (SII) and the superior parietal lobule (area PE); PE to retroinsular cortex (Ri) and rostral inferior parietal cortex (PF); PF and Ri to SII; and SII and Ri to the granular insula (Ig). SII also projects ahead of Ig to the dysgranular insula (Id). Finally, we have confirmed previously reported projections from Ig and Id directly to the amygdala and indirectly to the hippocampus via the perirhinal cortex and entorhinal cortex. These data thus demonstrate a somatosensory pathway that runs principally from SI through SII to the insula and from there to the limbic structures of the temporal lobe.

In the course of working out this ventrally directed cortico-limbic pathway for touch, we stimultaneously resolved a long-standing difficulty for the functional aspect of our hypothesis, which postulates that SI and SII process tactile information in sequence. It was supposed earlier that SI and SII were both projection zones of the primary somatosensory relay nucleus in the thalamus, n. ventralis posterolateralis, implying that the two cortical areas received and processed tactile information in parallel. Our new evidence has revealed, however, that SII receives its major thalamic afferents not, like SI, from the primary relay nucleus but from n. ventralis posteroinferior. This is a pattern of projections that is consistent with a sequential corticocortical pathway. As a further test of the proposal, we are currently examining the effects of ablation of SI on the somesthetic responsivity of neurons in SII. In support of the notion of sequential processing, we have found that the vast majority of SII neurons are entirely unresponsive to somatic stimulation after ablation of SI, and of the few units that remain responsive, fewer than 1% are activated by light tactile stimulation. These experiments are currently being replicated and extended to include recordings from the insula.

Since the insula has turned out to be an important link in the cortico-limbic pathway for touch, we have begun to examine the properties of insular neurons in awake normal monkeys. So far, over 500 neurons have been examined in the posterior two-thirds of the insula, which is the portion comprising the granular and dysgranular insular fields. Of the neurons sampled, 237 were later localized to the granular insular, and of these, 68% responded to innocuous somatic stimulation, whereas none responded to stimulation in any other sensory modality. In addition to their modality specificity, the most prominent characteristic of insular neurons was their large and often bilateral receptive fields. The properties of granular insular neurons in touch thus resemble those of area-TE neurons in vision and thus lend further support to the proposal that the cortico-limbic pathways in the two modalities are organized in a parallel fashion. One of the most critical tests of this proposal, however, remains to be performed, namely, a test of whether insular removals will impair tactile memory just as inferior temporal removals impair visual memory.

Nonlimbic structures and habit formation

On all of the memory tasks that have been described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals.

Soon after discovering this phenomenon in tests with objects, we found that the same dissociation, i.e. impaired recognition but spared discrimination learning despite 24-hour intertrial intervals, holds for pictorial stimuli as well. This paradoxical success in the presence of severe memory loss implies the existence of a powerful learning and retention mechanism outside the limbic structures of the temporal lobe.

We have since performed additional experiments to characterize further the essential difference in function between the limbic and nonlimbic mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research. For example, preliminary evidence regarding the effects of damage to area TE indicates that this cortical region is important not only for the limbic memory system but also for the nonlimbic habit system. Our aim now is to identify the subcortical structures belonging to the habit system, with our initial target being the corpus striatum.

Ontogenetic development of memory and habit formation

On the evidence that in the adult monkey there may be two relatively independent systems for learning and retention, we recently initiated a series of studies to assess the development of these two systems in infant monkeys. Results thus far indicate that visual recognition memory, as measured by performance on delayed nonmatching-to-sample, is absent in infants younger than four months of age and does not reach adult levels of proficiency even at one year. This slow ontogenetic development of recognition memory was shown even more strikingly with longer delays and lists. In sharp contrast, when 3-month-old infant monkeys were trained on object discrimination habits, they performed as well as adult monkeys in both acquisition and retention, even though intertrial intervals lasted 24 hours. These results strongly suggest that the two systems of retention that were found to be relatively independent in the adult monkey are also developmentally dissociable. Indeed, they provide evidence that the mysterious phenomenon of infantile amnesia could be due to the absence of a functional memory system in early childhood.

On the basis of this evidence, we have begun to prepare monkeys with neonatal removal of the limbic system (i.e. combined amygdalo-hippocampal removals) in an attempt to see how cognitive, emotional, and social behaviors develop in animals whose amnesia might persist from infancy through adulthood. Animals with neonatal removals of area TE are serving as controls. The results so far indicate that, at two and six months of age, monkeys with neonatal limbic lesions display abnormal social behavior, whereas the operated controls are essentially unimpaired relative to normal infants. At three months of age, neonatal ablation of area TE leads to a transient impairment in habit formation (compared to permanent impairment seen with the same lesion in adults), whereas limbic lesions in both infants and adults leave habit formation intact. Interestingly, data from both the normal and the operated infants suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males. At ten months of age, the infants with limbic lesions are severely impaired in memory formation, whereas the operated controls show significant functional sparing of the system (compared to the effects of TE lesions in adults). Our tentative conclusion is not only that early and late brain damage have different consequences but also that the degree and even the direction of the difference depends on whether the locus of injury is cortical or subcortical, the task measures habit or memory formation, and the subject is male or female.

Our plan is to follow the psychological development of our neonatally operated animals into adulthood. The results of the study will help to evaluate two provocative proposals from the clinical literature, namely, (a) that early dysfunction of the limbo-thalamic memory system could be one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology, and (b) that the reason a pure case of amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clnical picture of an amnesic adult.

In addition to the development of an animal model of childhood autism, we have initiated studies to determine more precisely the development of memory functions in infant monkeys. Our first attempt was to determine whether the visual learning measured by preferential-viewing tasks, which have revealed the presence of visual recognition ability in two-month-old human infants, is mediated by the memory system or the habit system. The results of the study indicate that visual recognition measured by this method develops in rhesus monkeys by at least 15 days of age; yet, despite being such a primitive process, it is markedly impaired by either early or late limbic-system damage. It is clear from this new finding that all limbic-dependent memory abilities are not delayed in development as we had earlier presumed. A hint as to why some may develop later than others has come from our concurrent neurobiological studies.

To complement the developmental behavioral experiments, we have been using the 2-deoxyglucose method to trace the development of the visual system metabolically. A series of monkeys with one hemisphere visually deafferented have been studied at various ages ranging from two days to six months. We found that, in all cortical visual areas of the intact hemisphere, glucose utilization was lowest in the youngest subjects, peaked at four months, and then declined in the six-month-old subject to levels found in adults. As a consequence, for each cortical area, the relative difference between the intact and deafferented hemispheres was smallest in the youngest animals and approached the differences seen in adults only in the four-month-old subject. The finding that adult levels of metabolic activity are not reached until about four months of age may help explain why the visual recognition ability measured by delayed nonmatching-to-sample does not develop until about this time. The recognition ability measured with preferential viewing, by contrast, although clearly dependent on a functional limbic system, may be less dependent than delayed nonmatching on the developing visual cortex.

Additional evidence that limbic and cortical systems may differ in rate of development has been provided by a new study on the neonatal distribution of opiate receptors in the rhesus monkey. A comparison between a newborn and adult brains revealed that the adult pattern of opiate-receptor binding is present at birth in allocortical areas and the hippocampal formation. These primitive areas showed conspicuously higher levels of opiate binding than the neocortex at both ages. In the neocortex, by contrast, marked differences were evident at the two ages. Whereas the adult brain was characterized by areal-specific laminar patterns in primary sensory, motor, premotor, and dorsolateral prefrontal areas, the newborn brain was not. In the newborn brain, only the primary visual cortex had a distinct laminar pattern of labelling; in all other neocortical areas the infragranular layers showed relatively high levels of opiate-receptor binding. Further, in the adult brain, polysensory areas (e.g. PG and TF) showed greater labeling density than modality-specific sensory areas, but this density difference was not apparent in the newborn brain. At the subcortical level, the density and pattern of opiate receptors in the infant and adult brains were similar, with a patchy mosaic of labeling in the striatum, high levels of labeling in certain amygdaloid and thalamic nuclei, and an absence of labeling in the mamillary bodies. The findings suggest that the distribution of opiate receptors in the macaque brain is adult-like at birth in limbic and striatal structures but is not yet fully developed in neocortical areas.

Summary

Through combined use of behavioral and neurobiological methods, we are beginning to discover some of the general principles along which the primate forebrain is organized to serve memory and other cognitive processes. (1) Each primary projection area in the cortex seems to be the source of two multisynaptic corticocortical pathways. Both pathways are composed of several cortical areas that are arranged hierarchically, one pathway being directed dorsally to the frontal motor system, the other ventrally to the temporal limbic system. Before reaching the motor system, the dorsal pathways from the several modalities converge in polysensory areas, which are critical for sensory attention, spatial perception, and motor guidance. The ventral pathways, by contrast, remain modality specific throughout their course and are important instead for stimulus recognition and stimulus meaning, and ultimately for triggering the motor response. (2) Stimulus recognition depends not only on stimulus processing along the ventral cortical pathway but also on storage of a central representation of that stimulus in the ventral pathway's last station, located in the anterior temporo-insular region. This region projects directly to the amygdala and indirectly to the hippocampus, and these two limbic structures project in turn to the medial thalamus, with further connections to ventromedial prefrontal cortex. All three of these regions (limbic, thalamic, and prefrontal) project to the basal forebrain cholinergic system, which innervates the entire cortical mantle. Storage of the central representation of a stimulus occurs only if this cortico-limbo-thalamo-prefronto-basal forebrain pathway is activated. Damage to this pathway results in recognition failure, a core symptom of amnesia.

(3) Once the central representation of a stimulus has been stored, it can enter into association with the stored central representations of other stimuli and other events, thereby providing the stimulus with meaning. Thus, stimulus-stimulus associations appear to depend on cortico-amygdalo-cortical interactions, while stimulus-affect associations probably depend largely on cortico-amygdalo-hypothalamic interactions. By contrast, stimulus-place associations seem to depend on cortico-hippocampal interaction; in this case the hippocampus may act as the site of converging information from both the ventral 'stimulus recognition' pathway and the dorsal 'spatial perception' pathway, the latter involving projections from the polysensory areas through the cingulate gyrus to the hippocampus. Finally, stimulus-act associations could depend on interaction, via the limbic system, between the ventral and dorsal pathways within the lateral prefrontal cortex, which interacts in turn with the motor system. (4) Destruction of the limbic portion of the memory system does not affect all forms of learning and retention, however. At least one form, which has been labeled habit formation, remains nearly intact, presumably reflecting the operation of a powerful cortico-nonlimbic mechanism for retention of stimulus-response connections. On the basis of behavioral and neurobiological developmental studies, this cortico-nonlimbic habit system appears to mature considerably earlier than the cortico-limbic memory system.

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Annual Report of the Laboratory of Developmental Psychology

National Institute of Mental Health

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Marian Radke-Yarrow, Chief

Summary

The research of the Laboratory of Developmental Psychology is concentrated in studies of the emotional and social development of children. Some of the investigations focus on the normative stages or features of development in these domains. In much of the work, emphasis is on environmental and constitutional factors that interfere with healthy development, and on factors within pathogenic environments that are protective or therapeutic with regard to children's psychological integrity. Because some children, regardless of environmental conditions, are emotionally and socially at risk, and others, regardless of conditions, are competent and resilient, research is directed, also, to an examination of apparent discordances in development. The children in our research are of three periods of childhood: the years of transition from infancy to childhood (1 1/2 to 4 years), middle childhood (5 to 11 years), and adolescence.

Before describing the specific research projects, some overall directions and considerations in the Laboratory's research will be mentioned. The family environment in which the child is reared receives major attention in research on the two younger age periods. This long-time area of inquiry is by no means a comprehensively researched one. We have been able to bring to it new and more powerful methods of investigation and conceptualization. Further, we have brought this area into psychiatric theory and research on major affective disorders, focusing attention on the implications of serious parental psychopathology (translated into rearing behaviors) for the environmental transmission of disordered behaviors in the offspring. In the studies of adolescents, biological considerations and extra-familial factors have received greater emphasis.

To the extent possible, research problems are addressed with a developmental perspective. That is, children are studied over time, either with continued monitoring or with follow-up designs. The research strategies (described later) are to obtain multiple indices of major variables and substantial samples of behavior under ecologically valid conditions. Therefore, a very considerable amount of staff time and expertise is required with each research participant. The projects of the Laboratory require expertise from a number of disciplines. Psychologists, psychiatrists, nurses, social workers, and endocrinologists are involved.

In the past year, reductions in available positions and in flexibility in staffing have put strains on the ongoing research. We have tried to meet emerging needs by developing skills in our current support-staff through in-service training and through the help of professional and technical staff supported from outside sources. Approximately half of the professional

and technical staff working on projects of the Laboratory are non-government-supported; also a number of projects are carried out collaboratively with universities. Descriptions of the specific research projects follow.

Studies of Adolescence

Investigation of adolescence is a relative new-comer in the program of the Laboratory. Two projects have been carried out and are now near completion. In their study of interrelations of biological changes and psychological functioning in early adolescence, Drs. Susman and Nottelmann, in collaboration with Drs. Loriaux, Chrousos, and Cutler (NICHD), have chosen a developmental design. Boys (ages 10 to 14) and girls (ages 9 to 13) were selected for each of five stages of pubertal development as defined by Tanner. Each child is followed at 3 times of measurement over a period of one year. Blood levels of gonadotropins, sex steroids, and adrenal androgens are obtained. Psychological measures include assessments of competencies, emotional states, relationships in the family and among peers, and problem behaviors. Parental attitudes and rearing practices are also measured. Data collection has been completed for all but a part of the third testing period. Data from the first testing period (when children are in the early years of adolescence but of different endocrine statuses) indicate clear and consistent relationships between endocrine status and aspects of psychological functioning in both the adolescents' self-assessments and the parental reports. For both girls and boys, hormone levels indicative of more mature endocrine status are related to feelings of low competence, low self-worth and negative self-image. For boys with the higher levels of sex steroids and adrenal androgens, mothers more often report obsessive-compulsive, delinquent, aggressive, hyperactive, schizoid and uncommunicative behaviors, and somatic complaints. For girls with the lower levels of adrenal androgens, mothers more often report anxiety, withdrawal, depression, aggression and cruelty. Early adolescence appears to be a period of high vulnerability psychologically. Within-individual changes in endocrine status and in behavior will be examined as data are analyzed from the second and third testing periods.

Adolescents are also the subjects of a study by Dr. Nottelmann. Here, using survey and interview methods, adolescents' coping with intrapersonal issues of self-definition and self esteem and with interpersonal relationships with peers, family, and society are investigated. A great many relations appear between adolescents' feelings about themselves and their overt behavior. For example, young adolescents with aggressive tendencies (bullying and picking fights with peers) (18% of the sample) have significantly lower self-esteem and see themselves as less competent academically than the nonaggressive adolescents. This difference is especially for girls. The total data set is being examined in terms of self-concept and its correlates. A second theme concerns the social support systems that are used in early adolescence. Age and sex differences appear. The younger group (6th graders) turn more to parents than to peers, especially the boys; the reverse is the case for the older group (7th graders), especially the girls. Mother is generally the person turned to; father is infrequently the person the adolescent turns to for psychological support, although both father and mother are sometimes named. An integration of findings from the studies is in progress.

Studies of Child Abuse

The development of physically abused children and characteristics of abusive parents are studied by Drs. Trickett and Susman. The research is based on the premise that both the causes of and the impact of child abuse can be understood only by considering physical abuse within the context of the enduring parent-child relationship or childrearing environment. Physically abused children ranging in age for 4- to 10-years and their parents were recruited from protective service agencies in the Washington Metropolitan area. A control group of non-abusing families, recruited from community agencies, was matched to the abusing families on age, race, and sex of the child and educational and occupational status of the parents. The total sample is 56 families. Standardized measures were used to assess the child's cognitive and physical functioning, social problem solving skills, and behavior problems. Emotional coping style and modes of relating to family members were assessed by observational methods. Assessments of the parents and the environment they provide for the child include tests and observations to determine parental frustration tolerance, parental childrearing attitudes, values and practices, characteristics of parent-child interaction, parental mood, and parental psychiatric diagnosis (SADS). Parents also kept records of naturally occurring incidents of discipline for five consecutive days.

Compared with the control group children, the abused children were reported to commit more antisocial transgressions (unprovoked aggression, destructiveness, out-of-control anger) and, in reaction to parental discipline, were more noncompliant and more likely to accompany noncompliance with negativistic verbal responses. This suggests both the contribution of the child to abusive incidents and the ineffectiveness of abusive parents' discipline attempts. Differences were found in the frequency with which abusive and non-abusive parents used different disciplinary approaches: Abusive parents were not only more likely to use punitive strategies, and more severe forms of physical punishment than controls, they reported significantly more anger after disciplinary incidents than non-abusive parents. Control parents were more discriminating in their disciplinary approach, using different techniques depending on the nature of their child's misbehavior while abusive parents tended to use the same punitive techniques regardless of the situation. Early analyses of parental psychiatric diagnosis shows a much higher proportion of diagnoses of depression (SADS) for abusive parents than for non-abusive parents.

Mothers in the child abuse study responded to an inventory probing their childrearing attitudes, values, goals, and practices. The Block Q-Sort, a well validated instrument, was used. This same instrument was used in another Laboratory study, of maternal depression. Abusive, depressed, and normal mothers were compared (Drs. Susman, Cummings, Iannotti, Trickett, Waxler, Yarrow). Abusive mothers reported pronounced and pervasive deficits in rearing. More than other mothers they valued harsh discipline, felt angered and disappointed by their children, showed deficits in emotional expression and regulation, lacked the capacity for providing enriching or stimulating experiences, devalued the child's opinion, and interfered with the child's autonomy. These findings disconfirm previous research reports suggesting that most interactions in abusive families are satisfactory and that problems are confined largely to incidents requiring discipline.

Mothers with major depression more often than normal mothers reported use of guilt-induction techniques and high levels of protectiveness. Mothers with current major or current minor depression were more inconsistent than normal mothers in their childrearing attitudes and practices. The inconsistency appeared to reflect carryover of depressive symptoms such as memory problems and helplessness into the arena of childrearing (e.g. the mothers forgot promises they had made to their children, and forgot threatened punishment and therefore did not carry through). Mothers with diagnoses of past depression were similar to normal mothers on a majority of their childrearing attitudes and practices, suggesting that many of the rearing problems of depressed mothers might be viewed as state- rather than trait-related.

The data also identify critical sex-linked differences in rearing experiences in deviant families. Deviations in parental practices were more likely to be directed toward girls than boys: Mothers reported more worry, more over-protective efforts, less enjoyment of the parental role, less open expression of emotion and less encouragement of openness to new experiences in their relation to girls than to boys. These tendencies were most pronounced for the mothers who themselves experienced emotional problems. Hence, girls from these troubled families may be particularly at risk for emotional disorders. The parenting problems reported by mothers of girls reflect environmental factors that may predispose girls toward feelings of helplessness, hopelessness, and lack of self-worth, which, may be precursors of adult depression--the incidence of which is two to three times higher in women than in men. Despite marked cultural changes in the past decade, attitudes and values regarding the socialization of boys and girls, based on the Q-sort data, seem to have remained much the same.

Parental Belief Systems Regarding the Origins of Child Behavior

How people perceive causes of events, particularly how much personal control they feel they have over their lives, has been widely investigated in clinical and non-clincial groups. Beliefs and feelings of helplessness have been shown to be associated with depression. Beliefs regarding personal control have not been examined in relation to mothers' perceptions of, and efficiency of, their parenting. Parents' conceptions of the causes of child behavior are especially relevant in studies of rearing by mothers who are clinically depressed. The mother's perception of the child's vulnerability to depression and her own feelings of control or lack of control over her child's development may well influence the rearing process. Clinical and anecdotal examples in which depressed parents have been given explanations or predictions concerning the likely mental health outcomes for their children demonstrate some very direct consequences of these messages to parents for how they care for the child and for the kinds of expectations they convey to the child concerning his/her own self and future. Affectively-ill parents who believe that nothing they do really matters, that their child's mental health fate is a "given", approach childrearing differently from parents with a less deterministic and simplistic outlook.

To investigate these issues systematically, an instrument has been developed which measures the structure of mothers' causal thinking about the origin of important aspects of children's behavior and personality (Drs. Kochanska, Yarrow and Kuczynski). For each behavior attribute, the mother indicates her beliefs as to the contributions of her own intentional behavior with the child, personality factors in herself, the child's father's behavior, genetics, and other factors beyond her control. Mothers also indicate the importance they place on each of the child behaviors and their satisfaction with the development of their children. Parents' causal theories will subsequently be examined in relation to the mothers' childrearing behaviors and characteristics of children's personality. This project is beginning data collection.

Early emotional-social development

A study of problem aggression in 2-year-olds (reported previously) is now in the follow-up period, when the children are 5-year-olds (Drs. Cummings, Zahn-Waxler, Iannotti, and Pierrehumbert). At both ages the children are observed under a variety of experimental conditions, in interaction with their mothers, unfamiliar adults, and familiar peers. Patterns of aggression, affiliation, empathy, and emotional regulation are measured. Cognitive assessments (the ability to take another person's perspective at 2 years and causal attribution measures at 5 years) are also made. Comparisons across time periods show significant consistencies: in mother-child relationships, in boys' aggression patterns, a positive relation between perspective-taking ability at 2 years and perspective-taking (empathy) at 5 years. Children of normal and depressed mothers do not differ on cognitive measures. Relative to children of normal mothers, children (2-year-olds) of depressed mothers were unlikely to engage in physical aggression with the playmates, likely to show preoccupation and upset when exposed to conflict and distress in others, and likely to be less spontaneous in their expression of emotions.

Emotional-social development in relation to rearing conditions

The remaining studies in the Laboratory are parts of one program project in which children's emotional-social development is investigated in the context of the family rearing environment. Families with and without parental depression are studied. This general research objective requires explication. Symptoms of depression such as sadness, agitation, anger, withdrawal, feelings of guilt, worthlessness, hopelessness and cognitive confusion, it is assumed, translate into rearing functions of discipline and control, teaching, stimulation through play, conversation, affectional relationships, etc. What are the translations, and what do they contribute to adaptive and maladaptive behavior patterns in the offspring? In research and theories relating parental psychopathology to offspring outcome, the interface of genetics and environment is rarely investigated; the processes between parents and children in the course of rearing are seldom observed directly. Also, there are scant data on the development of children's patterned emotions and social behavior. One essential in furthering research on these issues is a sensitive and precise instrument for measuring the rearing environment and the behavior of children in this environment. An effective research paradigm has been developed (Yarrow) which succeeds in obtaining detailed, natural observational data on parents' and children's behavior. The design (described in previous years' reports, MH 02144) brings parents and children to the Laboratory, an informal apartment, for a series of half days. The structure of these research sessions is such as to keep intact typical day-to-day functioning. The kinds of circumstances and activities that are sampled by the procedures are based on a conceptualization of the functions involved in rearing. The sessions are videotaped, thereby allowing detailed analyses. A second series of observations on each family is carried out 2 years later. Interviews and standard tests provide further data.

Families are selected on the basis of psychiatric assessment (SADS): parents diagnosed as normal, and mothers diagnosed as bipolar depressed, unipolar depressed, or minor depressed. Fathers' diagnostic status in the families with maternal depression is allowed to vary, except for the exclusion of schizophrenia or antisocial personality. Parents are interviewed to determine the presence of mental illness in other members of the immediate family and in first-degree relatives. Two children are studied in each family; they are 1 1/2 to 2 1/2 years old and 5 to 8 years old at the time of initial testing. Each child is given a psychiatric assessment at the time of the original testing and 2 years later. One-hundred-two families have been seen through the first series. Fifteen families have been seen thus far in the follow-up series.

Conversion of these raw data into concepts and coded entities has been the major analytic progress this past year. Coding systems have been developed that are conceptually and methodologically sophisticated and reliable. They are now being applied to the data on dimensions that we assume are critical rearing dimensions, and which may involve deficits or distortions when a parent is not functioning well. The dimensions include (a) the affective qualities of the environment and of parent-child relationships. From this coding system are obtained daily profiles of emotions expressed by parent and child, pervasiveness of given emotions, variety and lability, functions of emotions in relation to situations, specifically the socialization of affection, and mother's strategies for regulating and coping with child's emotions in the face of distress; (b) the determinants, content, and effects of parental discipline and control practices; (c) mothers as mediators of the child's cognitive development, i.e. the extent to which mothers impart cognitive content, the nature of the content, and the methods used to impart it; (d) verbal attributions by mother to her children, i.e. verbalizations directed to the child about the child, from which the child acquires knowledge and attitudes about the self.

Evaluations developed thus far of child behavior include measures of the child's self-control, compliance, initiative, social responsiveness, attentiveness, affective expression and regulation, caregiving and conflict behaviors, content of thought, play, coping strategies. The child's emotional bond to mother is assessed according to standard methods. The development of these instruments is the work of collaborations within the research group of Drs. Yarrow, Barrett, Cummings, Cytryn, Friedman, Iannotti, Kochanska, Kuczynski, McKnew, Sherman, and Waxler.

Each of the parental and child variables mentioned identifies a specific research project concerning rearing competencies and disorders in normal and depressed mothers, and/or correlates or consequences in healthy and deviant child behaviors. However, the ultimate objective of the program project is to deal with the patterns and interrelatedness of conditions and behaviors. At this point of progress, some findings can be reported that are preliminary. No attempt is made to relate them to each other.

(1) The quality of the young child's emotional bond or attachment to the mother is one of the most important influences on the child's general emotional and social functioning, and a predictor of later development. Of the many possible maternal variables that may interfere with the establishment of a secure relationship with her young child is the mother's affective state. Quality of the child's attachment was examined in relation to diagnosis of the mother. Based on a sample of 98 families, children of mothers with a major affective disorder have a higher incidence of insecure attachment (65%) than children of normal mothers (29%). The bipolar group has the highest occurrence of insecure attachment: 79% compared with 47% in the unipolar depressive group. In addition, there is a significantly higher incidence of very deviant insecure attachments in children of mothers with major affective disorders. Children of minor depressive mothers and normal mothers do not differ in attachment to mother.

(2) Based on the psychiatric interviews alone, more of the children of bipolar and major depressive mothers than of normal mothers show multiple behavior problems, i.e., are coded in multiple areas of concern across the spectrum of social, emotional, and cognitive functioning. More detailed assessments of the children are not ready for reporting.

(3) There are early indications of many process differences among mothers. How these differences map on to categories of depression and normality it is too early to know. One process difference is in the area of control. Success rates by mothers in gaining immediate compliance from their children vary widely. However, this is only one aspect of control; in many instances children as young as 2 negotiate with their mothers and attempt to work out compromises to come to some satisfactory resolution. To a significant extent it is in these communications between child and parent that one gains insights into the nature of the child's negativism and the child's learning of self-control, also insights into the nature of mother-child communication. There is suggestive evidence that older children 3- to 4-year-olds, of normal mother show increased competence in negotiation strategies, while children of depressed mothers tend to show no increase with age.

(4) The transmission of behavior through imitation has been examined. The imitative repertoires of 1 1/2 to 2 1/2-year-olds are extensive. Their imitations show development from simple repetitions to functional patterns of behavior. These include instrumental behaviors such as caretaking, self-care and household chores, interpersonal communication skills, mannerisms and expressive characteristics of the parent and verbal expressions. Imitations of parental discipline and emotions also increasingly appear in the child's interactions with others. Whereas younger children were more likely simply to repeat parental behaviors without directing them to others, older children are increasingly likely to direct their imitation toward others. Although most of the imitations consist of immediate repetitions, there is a significant increase in

the incidence of delayed imitations with age. This suggests that exposure to parental behaviors may have more lasting effects on children's memory and learning of new patterns of behavior as children grow older. The data suggest that imitation is an important process in children's acquisition of instrumental behavior, social skills, and emotional expression.

The content, amount, and occasions of mothers' verbalizations directed to the child differentiate another dimension of rearing. The amount of child-directed verbalization of some mothers is four times that of others. Most of the verbalizations carry cognitive (informational) content; fewer carry affective content alone. Many of the verbalizations convey implicit messages about the child's self; more of these are positive than negative. The verbalizations of a few mothers convey mixed messages to the child. These findings represent only the first descriptive account of analyses that will examine individual differences among mothers and consequences for children.

(5) Two kinds of psychiatric assessment procedures for children 1 1/2 to 5 years have been developed and are being evaluated. The first procedure relies on the child-clinician dyad in a semi-structured play interview consisting of segments of play with neutral toys, family toys, and toys of high aggression potential. Assessment is in terms of areas of concern (following a standardized set of categories) and of DSM III diagnoses. A second instrument brings together the strengths of clinical psychiatry and child development. Child is observed with mother in standard situations that involve conditions and requirements that are natural parts of the child's life (stress, closeness, pleasure, mother-unavailability, frustration, etc.). The situations are sequenced to comprise a reasonable script of events. The procedures can be carried out in the clinician's office. Behaviors are coded by the clinician; the analysis combines two types of codes: a prescribed set of coding judgments on a standard set of dimensions and clinical assessments. The assessments from the play procedure and the interaction procedure are being compared and evaluated against a number of sources of information about the child.

Plans for the next year:

The coming year will involve a major emphasis on analyses of the data of the first period of data gathering, and on collection of the data of the follow-up period. A year and a half is estimated for the remaining follow-up.

Professional activities of staff:

During the past year, investigators in the Laboratory have been active in the professional work of their disciplines. They serve on editorial boards of journals and on committees of professional societies; they have participated in symposia and paper sessions at national and international meetings; they have given invited lectures to university departments.

Research findings from the Laboratory have been reported this year in major media: public television, Norwegian Television, ABC Television, USA Today, APA Monitor, Newsweek, and U.S. News and World Report.

Annual Report of the
Laboratory of Psychology and Psychopathology
National Institute of Mental Health

October 1, 1983 to September 30, 1984

Allan F. Mirsky, Ph.D., Chief

This is the fourth full year report of the program of the Laboratory of Psychology and Psychopathology (LPP). In addition to the progress in research, which is reported below, there has been a major decision regarding a change in the LPP structure this year. The Clinical Infant Research Unit (CIRU) under the direction of Dr. Stanley I. Greenspan, will be transferred within the near future to the Bureau of Maternal and Child Health. Consequently the report of the CIRU will likely be our final one from that group.

The section on Clinical Brain Imaging (SCBI) continues to be a part of LPP and is headed by Dr. Robert M. Cohen.

A terminal report from the CIRU is appended to this Summary; the work of the CBI Section is summarized as a project report. We have occupied our permanent laboratory space in the ACRF since May of 1983; acquisition of our necessary research equipment is virtually complete and renovation of our laboratory has been accomplished. Although the present summary report is to some extent a recapitulation of last year's report, extensive progress has been achieved. We have made numerous contacts with organizations and persons both within and without the NIMH with respect to access to patient groups (schizophrenic, epileptic and brain-injured subjects), and we stand poised and ready to implement the human studies referred to below. The scope of the animal model studies is more restricted than had been planned; however, a number of studies of attention in the monkey are in progress and some have been completed.

A brief outline of studies appears below.

A. Human clinical studies of attention disorders

1. The taxonomy of attentional disorders
2. Brainstem mechanisms in attention impairment
3. Neurobehavioral studies in absence epilepsy

B. Animal models of attention disorders

1. Experimental models of generalized seizures of the absence type
2. Analysis of cellular activity in the attention system within the monkey brain
3. Brain lesion and state change effects on visual attention

C. Psychobiology of cognition

1. Studies of depression
2. Studies of dementia
3. Learning disabilities
4. Pharmacological studies of cognition

D. Autonomic nervous system activity in attention and psychopathology

E. Individual differences in the study of heredity and environment in schizophrenia

F. Cognitive, perceptual and nosological studies in depression

G. Clinical Infant Research Unit -- Final Report

Introduction

The basic new thrust has to do with the creation of a program of research aimed at developing a taxonomy of attentional disorders. We aim to describe in detail, at a number of levels of scientific analysis, the nature of the system within the brain that provides the substrate for attention, the basic organism/environment interaction. The system involves the participation of many elements within the neuraxis, from phylogenetically ancient structures within the mesencephalon and pons through structures in the diencephalon including some regions of hippocampus, through regions of the temporal, parietal, and frontal cortex. The program of research necessary to describe the attentional system and its manifold dysfunctions involves the study of a number of model or paradigmatic clinical conditions. These include patients with prefrontal, temporal, or inferior parietal lesions. Such cases represent perhaps the clearest instances of brain-behavior relation in that they involve the behavioral effects of specific lesions which can be documented anatomically with a reasonable degree of accuracy. In some of these cases, i.e., lesions including the right inferior parietal lobe, the attentional loss is most pronounced and obvious: the patient may neglect the entire left side of space contralateral to his lesion. The mechanisms underlying the loss, however, are not particularly well understood.

The next most difficult level of analysis involves study of patients with epileptic disorders of the generalized or absence variety. During the absence attack, which some of these patients display in relatively pure form (uncomplicated by even minor motor seizure manifestations), there can be total abeyance of consciousness for varying periods of time, up to a minute or longer. Such absence periods are usually accompanied by dramatic changes in the EEG: generalized, bilaterally synchronous slow discharges of the three per second spike and wave (SW) variety. These discharges allow a specific point of entry into the study of the impaired attention or consciousness; experiments can be designed to analyze behavior or stimulus processing during, preceding, and following SW discharges, so as to facilitate understanding of the nature of the pathological brain processes involved in the absence attack. The electrographic sign (the SW discharge) thus provides in some sense a temporary lesion which can be the subject of neuropsychological study. Although it is less satisfactory as an experimental variable than the existence of a documented loss of tissue, absence epilepsy does at least provide the opportunity to study each patient as his own control. The brain-injured and epileptic cases provide models and paradigms for the experimental analysis of attention loss which may help in the understanding of the symptoms of impaired attention in clinical states in which the neuropsychological nature of the disturbance is more obscure or not well understood. Instances of these include schizophrenia, infantile autism, and the various types of dementia (e.g., Alzheimer's disease, Korsakoff's syndrome, Huntington's disease). For many of the clinical states under study, the nature of the human clinical material ordinarily precludes the use of certain kinds of invasive experimental methods (e.g., deep probes or implanted electrodes, brain biopsies). Questions arise often as to the locus or nature of the lesion process in absence epilepsy, for example, which can best be approached, given current levels of technology, with animal models. Thus, the role of lesions or other perturbations in specific subcortical brain regions in absence epilepsy can be explored in animal models. Monkeys can be trained to perform complex attention-demanding tasks, and experimental modifications of the animal's brain can be performed: lesions, either static or irritative, of various subcortical structures; electrical or direct chemical stimulation; administration of neurotransmitters, hormones, enzymes, or blocking agents. The effects of such experimental agents in trained, behaving animals can lead to more refined hypotheses about the nature of pathophysiological processes; moreover, models for testing the efficacy of new therapies may be provided by this work. Aside from models of pathophysiology, however, the highly advanced and complex brain of the monkey affords an opportunity for study of the basic phenomena of attentional brain function in primates. A considerable program of work can thus be followed profitably, leading to analysis of neural processes, both at the level of the individual nerve cell and the nerve network, involved in attention. The broad outlines of projects which will be pursued by the LPP are described below in section B.

A. Human clinical studies of attention disorders

1. The taxonomy of attention disorders: A collaborative project

Many of the members of the LPP are involved in a joint project in which a wide variety of attentional, cognitive, autonomic, and electroencephalographically-derived tests are applied to the same populations of experimental and control subjects. These will include epileptic persons, schizophrenic subjects, brain-lesioned subjects, dementing subjects, and controls. The aim is to develop a profile of functioning for the several groups that will highlight the similarities and differences among them and will lead to a better neuropsychological characterization of their impairment.

There are four major elements within this program that will be discussed at this time: the work of the Cognitive Psychophysiology Unit (Dr. Connie C. Duncan-Johnson, head); the Imaging Studies of Dr. Richard Coppola; the Generalized Attention Test (GAT) development of Drs. Nakamura and Coppola; the development and implementation of the Neuropsychological Test Battery by Drs. Goldberg (consultant), Dr. Duncan-Johnson and Ms. Squillace. Dr. Duncan-Johnson's Unit will apply techniques of cognitive psychophysiology to elicit and evaluate the several event related potential components (auditory and visual) which have been shown to relate to attention, uncertainty and surprise (P300, N140, etc.). Similar experimental paradigms (e.g., the "odd-ball" method) will be applied to various groups of subjects with attentional disorders so as to be able to compare and contrast ERP component amplitudes, latencies and distributions. It is anticipated that these studies will help to illuminate (in conjunction with other methods) the nature of the similarities and differences among various attentional disorders.

Dr. Coppola's imaging laboratory became operational in the fall of 1982. Continuing from the work begun with Dr. Buchsbaum a 32 lead continuous EEG mapping system was implemented. The laboratory is now regularly recording 32 lead studies from a variety of patient groups. It appears that in some situations the additional imaging resolution gained from 32 leads vs 16 on one hemisphere can be quite useful. In other cases the 32 lead capability is used to record from both hemispheres simultaneously, allowing bilateral comparisons. Aside from evaluation of this new technique, studies are in progress examining patients with depression (under IV procaine HCL challenge); examining cases with presumed Alzheimer's disease; and developing EEG imaging in patients with epilepsy.

One of the behavioral measures to be used in this study is the Generalized Attention Test (GAT) which is being developed at the NIMH by LPP staff. It represents an extension and modification of the Continuous Performance Test (CPT) of attention which was originally refined and perfected at the NIMH during the period 1954-1961. The CPT has been used extensively by various groups of investigators, particularly in the study of epilepsy, schizophrenia, and metabolic illnesses. The GAT extends the concept of the CPT to involve a variety of cognitive dimensions (such as intra and inter-dimensional shifts), as well as parametric control of other perceptual variables. The GAT is expected to prove useful in exploring the dimensions of attention impairment in the several clinical groups under investigation in this taxonomy study. The GAT is designed for use with both humans and monkeys so that direct comparisons can be made of the results in each species. Preliminary results on humans indicate that evoked responses during performance on the GAT differentiate well between attention conditions. Also, monkeys have been successfully trained to perform on this task and some of the data from the first monkey subjects look to be extremely promising.

The Neuropsychological Test Battery was developed by various LPP staff, including Dr. Connie Duncan-Johnson, Ms. Kathleen Squillace and outside consultant Dr. Elkhonon Goldberg of Einstein Medical School. After considerable discussion, review of the literature and consultation with IRP staff, a battery of neuropsychological tests was devised which provides a reasonably complete assessment of the various executive, mnemonic, linguistic and attentive capacities of the human brain. The test battery has been administered to about 60 cases so far (in excess of 70, if one includes patients to whom partial test batteries have been given), and the information it provides will form the neurobehavioral data base which will be used to interrelate, evaluate and integrate the various electrographic, biochemical and other physiological measures applied to attention disorder patients.

2. Brainstem mechanisms in attention impairment

There are a number of lines of evidence leading to the inference that the classical brain stem systems implicated in attention (in the vicinity of the mesencephalic and pontine reticular formation) are damaged or malfunctioning in certain clinical syndromes. These include absence epilepsy, infantile autism, and possibly, some forms of schizophrenia. This project aims to use existing measures of auditory and somatosensory brainstem functioning which were developed for assessment of neurological (and especially demyelinating) disorders to assess the integrity of these structures in the several clinical conditions.

3. Neurobehavioral studies in absence epilepsy

In this project, specific sensory tests are used to assess functioning before, during, and following absence attacks in patients suffering from generalized seizures of the absence type. This work is done in conjunction with computer-assisted analysis of EEG changes prior to the appearance of SW discharge and has led to an appreciation of the changes in the energy (or more precisely the power) spectrum in cortical areas immediately preceding a paroxysmal EEG discharge. The findings already obtained in this project, which was begun at Boston University, may be considered as elements that can be incorporated in the development of a prosthetic device in treatment of some forms of epilepsy. That is, the EEG changes could be monitored and used to activate a device which would forewarn a patient of impending absence attacks. With help, patients might be able to develop strategies which could prevent, arrest, or abort such attacks. While it is true that most persons with absence seizures can be helped with relatively mild forms of anti-convulsant medications, it is not true of approximately 20% of such cases. These persons require either multiple anti-convulsant drug regimens or near-toxic doses of such drugs. Moreover, even low doses of anti-convulsant medications (whether considered to be easy to tolerate or not) may have deleterious side effects on behavior and biological capacities, especially when they are ingested by young, developing persons for periods of years. Excellent patient sources have been identified, and the cooperation of referring units has been secured. All of the hardware for this project has been secured and substantial progress is expected during the next year.

An additional component of this project to be pursued this coming year involves neurochemical studies. Neurochemical investigations of major psychiatric disease such as depression and schizophrenia have been disappointing. This may be for the reason that the causes of these diseases are heterogenous and not reflected in just one change in one neurochemical system. It may also be the case that the influence of thought and mood on neurochemical variables is rather subtle compared to the influences of other systems. The investigation of more homogeneous disorders which involve systems that are relatively better understood may offer more immediate rewards. Such studies may also help us to understand more traditional psychiatric disorders. Anorexia nervosa is one of these homogenous disorders; another may be absence epilepsy. (We have begun some neuropsychological studies of anorexia patients this year to specify their cognitive-attentive profiles). Approximately 20 have been studied, and a smaller group of controls. Cognitive psychophysiology has been performed on about six of these eating-disorder subjects. Pharmacological studies in absence epilepsy have found that medications that are clinically effective in preventing seizures act either on GABA or catecholamines. Absence epilepsy may involve a disturbance in the regulation and interplay between catecholamine arousal mechanisms and GABA inhibitory effects. This may be reflected in the "cortico-reticular" disturbance (as posited by Gloor) that underlies absence epilepsy. We intend to test this hypothesis in a number of ways. We will measure CSF during states of few absence seizures and during states of many seizures to determine if there are alterations in concentrations of catecholamines, GABA, and other related neurotransmitters. We will also attempt to influence absence generation by pharmacologic challenges of catecholamines. We are still attempting to recruit absence patients for these studies.

B. Specific animal projects

1. Experimental models of generalized seizures of the absence type

Over the course of the last 15 years, staff now in the LPP have been interested in the development of techniques for simulating the generalized seizure patterns, as well as the behavioral accompaniments, of absence epilepsy. The tradition of this type of model dates back to research by H. H. Jasper and colleagues (e.g. Hunter, Perot, Ingvar, Pollen, Weir) at the Montreal Neurological Institute beginning in the 1940's. These earlier studies depended for the most part on electrical stimulation of various brain regions, mostly in the midline structures of the diencephalon and mesencephalon. This work led to the concept of the "centrencephalic system" as described by Penfield and Jasper and antedated the "reticular formation" by some years. It also influenced the thinking of many brain scientists about the cerebral bases of attention and consciousness. The neural bases of these behavioral states is still obscure; however, we are still impressed by the potentially close relationship between the brain system involved in generalized seizure discharges and that which is responsible for the maintenance of consciousness. Animal models thus remain important investigative tools in this research effort. We have explored the use of electrical, chemical, and metabolic methods of inducing discharges. In our laboratories, we have used in past studies: electrical stimulation of subcortical and cortical structures, administration of chlorambucil and pentylenetetrazol, subcortical implantation of aluminum hydroxide cream, and application of conjugated estrogen symmetrically to forebrain cortical surfaces. Most recently, we have done some experiments with compounds which block metabolism of GABA (i.e., gamma-vinyl GABA) and produce the paradoxical effect of inducing symmetrical and synchronous SW-like activity in the EEG of the monkey.

Stimulation of the mesencephalic reticular formation (MRF) and related areas in the monkey produces absence-like behavioral symptoms. The MRF is known to have far-reaching ascending influences which are likely to be the basis of the absence symptoms. These ascending influences are poorly understood in the monkey since more accurate work with radioactive amino acids as tracers has not been done. We have therefore begun a collaborative study with Dr. Linda Porrino (LCM) of the diencephalic terminations of the MRF. One animal has been prepared for study of the various connections of the MRF.

Such models of generalized seizure activity permit the testing of various hypotheses and provide an experimental tool for study of single unit activity (as an example) under conditions of seizure.

2. Analysis of cellular activity in the attention system within the monkey brain

The concept of an attention system within the brain has been alluded to in various portions of our annual report. One could adduce evidence for the viability of this concept from a number of approaches or lines of evidence: the effects of lesions and other neuropathological conditions in human subjects; experimental lesions or other manipulations of the brain in animal subjects trained on tasks requiring or designed to accentuate attentive behavior; anatomical-physiological considerations. All of these lines of evidence appear to converge in suggesting that there is a system within the brain that is specialized for the attentional needs of the organism. One way of characterizing it is to study the attributes of individual nerve cells in those brain regions that allegedly form part of the system. We have conducted studies of two such brain regions to date: the meso-ponto-diencephalic portions of the brainstem and the prefrontal cortex, including both lateral and medial portions. Both of these locations contain large numbers of a particular type of cell which appears to have a specialized function with respect to attention. Our method of identifying this cell type has been based upon an experimental test paradigm in which animals are trained to perform in a go, no-go visual discrimination task. Attention-related cells are those which fire in both go and no-go trials. That is, they fire on trials when the animal has merely to attend to a stimulus display and performs no obvious motor response except holding a paw on a response key (no-go trials); they fire as well on trials on which a brisk lift and hit response to obtain a fruit juice reward is necessary. The cellular response may be identical on both trial types and may in addition anticipate the onset of the cue stimulus by several hundred milliseconds. This type of cell we have labeled as type II and, as noted above, is found distributed throughout certain regions of the brain stem and prefrontal cortex. Further studies will be aimed at providing a systematic functional analysis of this cell type, searching for other loci in the brain where they may be found, characterizing the relation among the several loci, and beginning pharmacological studies of these cells. As is documented in project report Z01 MH 00506-04 LPP, a gradient of type II through possibly transitional cells through type I cells has been described in certain regions of the monkey frontal lobe. This work suggests that there may exist in the premotor area of the monkey a transitional cell type between the "attention" cell and the motor execution cell. This cell type may serve some bridging or connecting function between the attentional and motor execution systems.

3. Brain lesion and state change effects on visual attention

There is collaboration with LN and LCM in some parts of this work. One of the goals has been to specify the role of non-visual regions of the primate cerebrum in visual attention, and this work has used several techniques including lesions, stimulation, and electrographic recording of brain potentials under various lesion and non-lesion conditions. Other studies have been concerned with the recording and analysis of visually-evoked potentials under a variety of perceptual conditions, and with drug or sleep state effects on the mechanisms of visual attention.

C. Psychobiology of cognition

The aim of this research has been to relate psychological and biological determinants of various components of cognition that are involved in the acquisition, processing, retention, consolidation, and retrieval of information or experience.

Experiments have been designed to characterize and contrast cognitive failures in patients with disturbances of mood, children with various forms of impairment in learning, and populations of neuropsychiatric patients including those with Huntington's disease, Korsakoff's psychosis, and Alzheimer's disease. How are specific forms of central nervous system changes related to discrete alterations in cognitive processes? Components of information processing in unimpaired individuals are also studied. A third type of study of both unimpaired individuals and patient groups uses various types of drug manipulation to alter specific aspects of cognitive processes. How do drug treatments that alter the activity of discrete aggregates of neurons relate to qualitatively different changes in cognitive processes?

1. Studies of depression

We can now define with some precision the kinds of cognitive processes that are impaired as well as those that are left relatively intact in patients with disturbances in mood (depression). Depressed patients are impaired in some but by no means all aspects of learning, memory, and retrieval. In part, this is a function of the intensity of their depression; moreover, the retrieval of experience is in part mood state-dependent. However, some aspects of information processing are particularly likely to be interrupted. Using models that distinguish between automatic versus effortful (or passive versus active) processing in cognition, we have noted that depressed patients may be profoundly impaired when cognitive processes require active or effortful as opposed to passive or automatic kinds of operations. For the latter, the depressed patient may be indistinguishable from a normal control. In fact, the extent to which depressed patients are able to manifest effort is highly correlated with the intensity of their depression and with the extent to which they can perform these non-automatic active cognitive operations. Also, the extent to which depressed patients are provided with organization of structure (as opposed to random uses) in information-processing tasks is the extent to which they are relatively indistinguishable from normal controls in terms of learning and memory.

How depressed patients think about information appears to determine many aspects of their problems in learning and remembering information. In concept learning, depressed patients develop overgeneralized hypotheses and do not "appreciate" disconfirming feedback. Their thinking appears to be global or overgeneralized and therefore, their processing of new information results in the formation of poorly elaborated weak memory traces. This can also be seen in how they process emotionally salient vs. "neutral" information and other stimulus characteristics that should ordinarily lead to effective information processing.

Recent studies in patients with Parkinson's Disease (PD) further highlight the distinction between impairments in automatic and effortful cognitive operations. Early stage PD patients experience memory-learning failures on tasks that require effort but not those that can be accomplished automatically. The memory-learning pathology is very much like that evident in depressed patients and unlike that seen in progressive dementia patients of an Alzheimer's type. We have also been able to show that mood changes along with changes in personality configurations (as seen in multiple personality syndrome) affect changes in how experiences are encoded and organized and also how they are retrieved from memory. Those cognitive changes are similar to drug related state-dependent learning and state-dependent retrieval effects.

2. Studies of dementia

Patients with progressive idiopathic dementia (most probably of the Alzheimer's type) have classically been diagnosed on the basis of the cognitive state that they present clinically. These patients demonstrate profound impairments in learning and memory as well as other kinds of cognitive operations. We have now described more fully the characteristics of the cognitive dysfunction in these patients and defined some of the mechanisms that determine their learning/memory failure. In a study of a large series of early stage Alzheimer's patients, we have demonstrated that information is lost from memory relatively rapidly, that immediate memory is relatively unimpaired, and that any type of learning/memory operation that requires the establishment of permanent trace events is dramatically disrupted. These impairments are due in large part to processing or acquisition deficits which then result in weak trace formation and, therefore, failures to remember. Cognitive theorists have argued for a distinction between semantic memory (that is, the repository of information about how knowledge is organized or structured) vs. episodic memory, or the memory for ongoing, recent events. Although these two kinds of memory have been viewed traditionally as being separate and distinct, we have found that there is an important linkage between the two, one that defines in large part the characteristics of the memory impairment in Alzheimer's patients. We have shown that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results can have important implications both diagnostically, in distinguishing this group of patients from other groups, as well as for potential treatment strategies.

Cholinergic drug treatment of progressive dementia patients has proved to produce weak enhancement of some components of information processing. It would appear that those patients who are least cognitively impaired are most likely to benefit from such treatment. In contrast, vasopressin-treated progressive dementia patients have shown temporary marked changes in learning and memory, particularly in those subjects who can access previous knowledge better, following such drug treatment.

The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies to distinguish between these two groups clinically. Three dimensions that are particularly useful in distinguishing the cognitive impairment in depression from that of the progressive idiopathic dementia patient are: (1) that of automatic vs. effortful processing; (2) the degree to which effort is extended in accomplishing tasks; and (3) the extent to which the information being processed is either random or highly structured.

3. Learning disabilities

We have examined cognitive changes in children with various forms of learning disability. Two groups of these children have been studied: in one, hyperactivity is part of the syndrome; in the second, there is no evidence of hyperactivity or generalized retardation. All of these children demonstrated dramatic impairments in learning and memory that resemble the kinds of cognitive losses seen in some groups of adults. The resemblance is closest to depressed patients, but it also mimics the kinds of cognitive changes that are produced by drugs which disrupt cholinergic and noradrenergic activity. Thus, both hyperactive and learning-disabled children demonstrate impairments in effortful processing of information, whereas automatic processing is left relatively intact. In fact, on our incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children are also impaired in those characteristics of cognition that require the imposition of organization in memory.

4. Pharmacological studies of cognition

Recent studies have shown that the dopamine system is involved in those types of cognitive operations that require effort but not for cognitive processes that can be accomplished automatically. When elderly unimpaired subjects were treated with L-Dopa/carbidopa their recall of information was enhanced (effortful processes) but their memory for how often an event took place (automatic processing) was unaffected. This apparent specific role of the dopamine system in modulating some types of cognitive processes is important both for what it teaches us about the psychobiology of cognition and its potential for the development of therapeutic strategies that would reverse cognitive impairments.

We have also begun to explore the role of the serotonin system in cognition. We have been able to show that zimelidine, a relatively specific 5HT reuptake blocker, can substantially reverse the cognitive impairing effects of acute doses of ethanol in man. Ethanol disrupts the memory for weakly processed information and zimelidine reverses this acute amnesic-like effect. It would appear that the serotonin system may serve to amplify weak memory traces.

In both patient groups and unimpaired subjects, we have found that cholinergic antagonists and agonists produce cognitive effects that are qualitatively different from those of drugs that have their major action on catecholamine activity (either as agonists or antagonists). A series of studies has been completed demonstrating the role of neuropeptides, such as synthetic vasopressin-like substances, in enhancing aspects of learning and memory. The enhancement occurs not only in cognitively impaired depressed patients but in unimpaired subjects and in patients with progressive idiopathic dementia as well. The general pattern of these findings implies that different neurotransmitter systems and/or neurochemical mediators may be involved in the regulation of various aspects of the acquisition, retention, and retrieval of information.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that: (a) cholinergic antagonists, such as scopolamine, mimic many of the characteristics of progressive idiopathic dementia; and (b) combinations of cholinergic agents produce small but reliable enhancements of some aspects of learning and memory in such patients. The amount of enhancement in learning and memory is limited by the degree of cognitive intactness of the patients. While alcohol has been viewed traditionally as one type of pharmacological manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory (in collaboration with NIAAA) has demonstrated that post-processing manipulation, including treatment with alcohol, can produce some enhancement in learning and memory. This paradoxical improvement, as well as that evident in the effect of vasopressin on reversing retrograde amnesia following administration of ECT, implies that it is important to continue to explore the biological events that succeed information processing.

D. Autonomic nervous system activity in attention and psychopathology

The central focus of this research is the role of attentional processes and autonomic nervous system (ANS) functioning in psychopathology, especially schizophrenia. Studies are directed toward several basic issues: (1) the nature of the attention and ANS dysfunction, (2) the diagnostic specificity of the dysfunction, (3) state vs. trait issues, (4) the neurobiological basis of attention and ANS functioning.

In a completed, but as yet unpublished study, we have replicated earlier findings that a pattern of high baseline ANS activity, slow adaptation and habituation, and a sluggish ANS response to stress were associated with a schizophrenic diagonsis. We have examined ANS activity in different subgroups of patients. Only unremarkable differences were found between patient groups who improved, worsened, or showed no effects from IV dextroamphetamine compared to a placebo infusion. However, significantly reduced ANS responsivity to significant stimuli and situations were found in schizophrenics with cortical atrophy compared to patients with normal CT scans. Since the ANS results for the patient groups differed from control values in the same direction, the data suggest a quantitative rather than a qualitative difference between groups contrary to speculations that have been advanced elsewhere. In a current study on schizophrenia, a major aim is to compare several RT methods of assessing attention deficits in order to define more precisely the nature of attention impairments in schizophrenia and to investigate the role of ANS activity in attention impairments.

Diagnostic specificity of the measures is being investigated by testing patients with a major depressive illness, obsessive-compulsive neuroses, and panic-anxiety disorder and adults with a history of infantile autism on part of the schizophrenia protocol. Comparisons of obsessive-compulsive adults with controls indicate higher baseline ANS arousal in the patients but no differences in habituation. In a group of adolescent obsessive-compulsives, only the boys showed this pattern of results compared to matched controls. Obsessive girls had low arousal and ANS responsivity. Hopefully, correlations with other biological markers will shed light on this puzzling result.

Our prior work suggested that many ANS variables represent stable traits that may be partly genetically determined. We are continuing to study their relationships to such personality variables as sensation seeking, extraversion, and psychoticism in normal subjects and are collaborating with investigators who are obtaining biological and neuropsychological measures on the same subjects. State influences are being studied in patients diagnosed as multiple personality. Nine patients and five controls have been tested in four or five sessions in a design which enables comparison of within personality with between personality variations for each subject. Results show that eight of nine patients had significant day-to-day consistency in between-personality variations in reaction time and in more of the ANS variables than would be expected by chance. Controls produced reliable consistency only in hypnotic states. In patients, there was a partial carryover of habituation from one alternate personality to the next almost as large as that seen in the controls. This suggests an influence of the experience of one personality on the ANS reactions of others. The relationship of this to conscious awareness among alternate personalities will be investigated on a case-to-case basis. In another study related to state influences on ANS, normals are tested when they are either supine or standing. Standing doubles plasma norepinephrine and our results show it elevates electrodermal and heart rate base levels and increases ANS orienting responses to tones in frequency and amplitude. Effects on two-flash threshold differed for subjects differing in "psychoticism". Standing seems to increase ANS activity, without confounded changes in attention or distraction, and it could have wider application in studying the effects of "arousal" on behavior. Our results also show inverse correlation between ANS activity and psychoticism, suggesting that psychotocism may be more akin to sociopathy than to clinical psychosis.

Neurobiological bases of attention and ANS activity are studied by investigation of the effects of drugs with fairly specific actions. Analyses will begin shortly on the comparative effects of several drugs on schizophrenia including pimozide, a dopamine receptor blocker, and propanolol, a beta receptor blocker. The effects of prazosin, a specific alpha noradrenergic antagonist, in schizophrenics were an increase in ANS arousal but no clinical changes. The ANS effects are likely due to an increase in plasma NE. The results confirm our previous conclusion of an independence of variations in ANS activity and in psychosis. A recent study compared the effects of an MAO type A inhibitor (clorgyline) with clomipramine, a tricyclic antidepressant, in obsessive-compulsive patients. Although only clomipramine was clinically effective, both drugs reduced electrodermal base levels. However, only clomipramine reduced ANS responding to simple tones and task related stimuli. This result is of interest because ANS hyperresponsivity has been implicated as an etiological factor in theories of obsessive-compulsive disorder. Moreover, the patients with the greatest clinical improvement showed the largest drug effects on several of these variables.

The ANS effects of a chronic (2-week) period of high dietary caffeine consumption vs. placebo in normal children was investigated with Dr. Rapoport using a design that also permits testing the effects of habitual caffeine use. Results replicated previous studies from this project using both acute and chronic caffeine doseage in finding increased ANS arousal indices and retarded habituation in subjects taking caffeine. Also confirmatory were findings that low users had higher arousal indices on placebo compared to habitual users. This suggests that the characteristic level of ANS activity of a child may, in part, determine the propensity for caffeine use. Since caffeine affects adenosine and benzodiazapine receptors, the data suggest that these systems are involved in the control of ANS activity.

E. Individual differences in the study of heredity and environment in schizophrenia

The project is composed of the following studies:

1. An intensive multidisciplinary study of a family with monozygous quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome.

The first study of this family was completed and published in book form in 1963. We have continued our contacts with this family to follow the clinical course of these women and to see how the course is related to earlier and current life experiences. A second intensive multidisciplinary study of these women was completed, so far as data-gathering was concerned, in June of 1981.

This study includes a number of the same variables that were examined in the quadruplets in the late 50's (e.g., CPT, reaction time, autonomic nervous system arousal and habituation) and adds a considerable number of new methods and techniques for assessing behavioral and biological factors. Among the new methods are the CAT scan, the PETT scan, far-field brainstem auditory evoked potentials, cerebral mapping of various EEG components and measures of many biogenic compounds from blood, urine, and CSF whose existence was unknown or for which no link to psychopathology had been established twenty years ago. The focus of this effort remains the same as it was in 1963: to help illuminate the differences in the severity of illness among these women. We have added, where possible, studies of the mother of the quadruplets, of the husband of the one who has been married, and of the two sons who have issued from that marriage. This additional information may also shed some light on possible genetic factors in schizophrenia. The data have been analyzed fully and are in press in Psychiatric Research.

2. Studies of adoptees with schizophrenic parents and their biological and adoptive families

This represents a completion of work done with a cohort of adoptees and controls that were obtained in Denmark. Although a considerable portion of this work has been published, much of the psychological information was not analyzed. This is in the course of being completed at this time. In addition, a reanalysis of the original case material has been completed and has appeared in the American Journal of Psychiatry. Several other papers are in preparation.

3. A study of children of schizophrenic and control parents reared in town or kibbutz in Israel

This study, begun in 1965, has involved a multidisciplinary (psychological, psychiatric, neurological) examination of a cohort of 50 children with schizophrenic parents together with 50 matched controls. Half of each group was raised on a kibbutz, and half was raised with their nuclear families in cities and towns in Israel. The children have been seen twice: once in 1966-7, when they were about 10 years of age, and once in 1973, when they were about 17. Aside from several reports of the neurologic findings (suggesting more "soft" signs in the index cases) none of the work has been published. Major progress has been made this year: (1) A series of manuscripts has been prepared describing available aspects of the 1966 and 1973 studies. In addition, major portions of the data have been analyzed. These efforts will form the basis of a comprehensive report to be published in a single issue of the Schizophrenia Bulletin. This report is scheduled for publication this year. (2) In 1981, a third study of the original Israeli cohort was begun, and has now been completed. The results are startling, since they suggest that being raised in a kibbutz environment is more likely to lead to major psychopathology, given a schizophrenic diathesis, than is growing up in the nuclear family within a city environment. This followup report is being included as part of the comprehensive report in the Schizophrenia Bulletin. Some of the results were presented at the 1983 meeting of the American Psychiatric Association.

The objectives of all of these projects are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

F. Cognitive, perceptual, and nosological studies in depression

This project has involved a variety of studies, of which two are still being completed. All are at the stage of final data collection and are also at or near the completion of data analysis. Progress over the past year will be summarized below, while more detailed descriptions appear in the individual project reports. Since these projects were under the direction of Dr. Edward Silberman, who left the LPP, no additional data are being collected. It is expected that all the projects will be concluded shortly. Dr. Silberman remains a Guest Worker in the laboratory.

1. Psychomotor and psychosensory symptoms in affective illness

Data have been collected on a total of 111 patients with affective illness, partial complex epilepsy, and hypertension (controls). Preliminary analyses indicate that affective and epileptic patients are indistinguishable in frequency of certain kinds of visual, auditory, visceral, and cognitive illusions and hallucination, while paroxysmal motor phenomena are present only in the epileptic group. The relationship of such symptomatology to underlying personality factors and life course of illness in the affective group is now being analyzed.

2. Lateralized hemispheric function in depression

Depressed female subjects have been found to show right hemisphere advantage in a verbal processing task which normally displays left hemisphere advantage. Since such a task is an obligatory left hemisphere task in normals, these results suggest shifts of hemispheric function as a concomitant of the depressed state. Further studies involving male depressed subjects and depressed-recovered patients are now being planned. A manuscript has been submitted for publication.

G. Clinical Infant Research Unit - Final Report

The Clinical Infant Research Unit (CIRU) is currently completing a preventive intervention clinical service demonstration program for infants and their families, begun as part of the mission of the Division of Mental Health Service Programs. The CIRU is formulating a new agenda in keeping with an imminent planned transfer to the Division of Maternal and Child Health, HERSA, where the CIRU will continue to develop and evaluate diagnostic and intervention strategies for at-risk and disturbed infants and their families. Because this Unit is in transition, a brief narrative describing its background will be substituted for the traditional project report.

The program of the CIRU began over seven years ago. In addition to offering preventive intervention services to multi-risk families and their new infants (beginning prenatally) assessment was made of a group of normal families and babies. The main questions and hypotheses were:

1. Would it be possible to identify reliably a range of psychopathologies quite early in infance in both the infants and their families?
2. The patterns of psychopathology identified would include affective, cognitive and physical-neurological dimensions. It was assumed that intensive clinical work (stemming from psychodynamic, developmental and structural perspectives) including observing behavior in a therapeutic relationship, would reveal the most complete picture of the patterns of psychopathology in these infants and their families, the potential reversibility of these patterns and the techniques that on a case-by-case basis appeared useful.
3. The patterns observed would lend themselves to a new classification

system of psychopathology in infancy and early childhood.

4. Case-by-case work with each infant and family would suggest the range of service system strategies and clinical techniques that would be required (e.g., the failures would teach as much as the successes). This approach would indicate, as well, which clinical techniques and provisions of service were most effective for the types of problems encountered.

5. Early forms of psychopathology would be partially or completely reversible, when access to the family and baby could be maintained and individually tailored clinical techniques used.

6. Severe infant and family patterns of psychopathology would not be reversed when access to the family was not possible for long periods of time.

7. When the "level of therapeutic relationship" achieved (using a therapeutic process scale) was controlled, there would be functional relationships between prenatal, perinatal and early developmental patterns as well as later developmental patterns in physical, cognitive and affective domains with the strongest relationships reflected in the affective functioning of the children and their families.

8. The level of therapeutic relationship attained would predict outcome at ages 3-5 more strongly than family or infant variables.

The CIRU worked with 50 multi-risk families and their infants, 20 teenage mothers and their infants and a small group of "normal" families and their babies. During the program a new instrument (the GLOS) was developed to assess reliable infant and caregiver affective adaptation and maladaptation. Studies of interrater reliability indicate that GLOS behaviors can be rated quite reliably by new raters. In addition, studies of infant and caregiver behavior over short time intervals indicate that the GLOS-measured bheaviors (in terms of both group means and individual differences and profiles) are highly stable in the early, middle, and latter part of the first year of life. In addition, there were age differences reflecting developmental progression based on both the developmental structuralist model and the existing literature on emotional development in infancy.

Patterns of psychopathology were identified in the infants as early as the first month of life (difficulties with homeostasis) and group differences between families receiving intensive intervention and those receiving evaluations, consultative crisis intervention and referrals to community agencies were also evident by 1 month of age. Interestingly, the "multi-risk" families who were recruited because of problems with child rearing were also found to be characterized by factors associated with a high degree of psychiatric disturbance. The literature usually describes such families according to attitudinal value, motivational and service usage problems; over 30 years ago it was estimated the multi-problem families constituted 6% of the population that used over 70% of the public mental health, social service, and health services.

Most important, however, were the findings from this study regarding the range of psychopathology that could be described in the early years of life. These included difficulties with using the senses for self regulation and taking an interest in the world, forming an emotional interest in the human world, learning "cause and effect" type affective signaling, and difficulties with developing, elaborating and differentiating symbolic or representational capacities. For each infant pattern we were also able to identify maladaptive caregiver patterns. These findings which are descriptive in nature and therefore difficult to present are briefly described in detail in our monograph, "Psychopathology and Adaptation in Infancy and Early Childhood; Principles of Clinical Diagnosis and Preventive Intervention." New diagnostic scales and clinical rating formats have been developed.

Equally important were the findings regarding the service system and clinical approaches that these families required, also described in the monograph mentioned above and in a new book of case studies entitled "Infants in Multi-Risk Families."

Analysis of the 3 to 5 year old outcomes are in progress. Preliminary trends suggest, however, that early psychopathology is highly reversible when there is continuing access to the families.

Functional relationships have been found between the therapeutic level attained in the intervention program and emotional adaptation in the infants and young children. In addition, relationships have been found between prenatal caregiver patterns, developmental progress, and various outcomes.

In a collaborative study on a non-intervention sample of multi-risk families, using the CIDP multi-risk criteria, a relationship has been found between the number of risk factors early in life and poor performance on measures of intelligence and social adaptation. For example, the likelihood of children of families with multiple risk factors of falling into a low IQ group (50-85) at age 4 was 25 times as great as those in the low-risk group.

The CIRU is currently developing additional diagnostic tools to assess adaptive and maladaptive sensory, motor, and affective functioning in the first three years of life. The future goals of the CIRU will include refining diagnostic and intervention technologies in the context of developing and implementing a clinical model for community-based comprehensive intervention services for infants and families at risk for developmental and emotional delays or disorders, including those involving sensory, motor, cognitive, affective and family functioning.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
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Melvin L. Kohn, Ph.D., Chief

The Laboratory is now so small that its long-term viability is threatened. For the present, though, we continue to thrive. This has been another highly productive year, both for our long-standing research on the relationship between job conditions and adult psychological functioning and for our increasing focus on the relationship between social structure and the psychological development of children. As in recent years, our cross-national comparative studies, in collaboration with sociologists from Poland and Japan, have greatly strengthened the Laboratory's research program.

During the current year, the Laboratory's major research efforts have been addressed to the following activities: an analysis of the processes by which values are transmitted from one generation to the next; further analysis of the relationship between educational experience and children's personality development; the beginning of an analysis of intra-family dynamics and children's personality development; an assessment of the impact of husbands' and wives' social statuses on wives' psychological functioning; continued cross-national comparative analyses, for Poland and Japan, of the relationships between job conditions and adult psychological functioning; a participant-observational study of the experiences of the deinstitutionalized mentally ill; and continuing methodological inquiry. This Annual Report will summarize all of these activities.

THE INTERGENERATIONAL TRANSMISSION OF VALUES

The goal of Melvin Kohn, Kazimierz Slomczynski, and Carrie Schoenbach's analysis is to redefine a classic sociological problem -- the transmission of values in the family. By values they mean conceptions of the desirable -- criteria of preference. By parental values, they mean the values that parents would most like to see embodied in their children's behavior -- the characteristics they consider most desirable to inculcate in their children. Parent-to-child value transmission implies, at minimum, that children come to hold the same fundamental values for themselves that their parents think desirable for them.

The investigators see the transmission of values in the family as part of a much more general process by which social structure -- in particular, social stratification -- affects children's values and orientations, with the family as only one -- albeit a crucial -- institutional mechanism in this process. As described in last year's Annual Report, the investigators began their analysis of the process of value transmission by developing confirmatory factor-analytic measurement models of parents' and children's values, using data from representative samples of parents and children in the United States and Poland. In both countries, the focus is on valuation of self-direction versus conformity to external authority. From these analyses, it appears that the correlations between parents' and children's valuation of self-direction/conformity are considerably stronger than past studies would have led one to expect. For the United States, using longitudinal data for fathers and considering values for children across the entire age-range of 13 to 25 years of age, they found the correlation between fathers' and children's values to be .58 and that between mothers and children to be .52. The analysis for Poland uses cross-sectional data and deals with children in the younger half of that age-range. The correlation between mothers' and children's valuation of self-direction is nearly identical in Poland (r=.55) to what it is in the United States. The correlation between fathers' and children's values, however, is lower for Poland (r=.37) than for the United States. It is not yet known whether this is a true cross-national difference or a methodological artifact -- since the U.S. analyses not only deal with a much broader age-range of children, but also are based on longitudinal data for fathers, which may strengthen the measurement properties of the index of paternal values. This is an issue that will be explored further.

Even correlations of .58 and .52 leave much to be explained, with fathers' and mothers' values together "accounting for" less than half of the variance in children's values. Moreover, calculating the correlations between parents' and children's values is only the first step in the analysis of the intergenerational transmission of values. Even a very high correlation between parents' and children's values does not necessarily mean that parents' values have been transmitted to their children. A full analysis requires the development of causal models that assess the degree to which social stratification affects children's values through its effects on parents' values (then transmitted from parents to children) and the degree to which social stratification affects children's values (if it does) through other processes. Answering this very general question requires answering the following constituent questions:

1. What are the contributions of husband's and wife's educational attainments, occupational statuses, and incomes to the social stratification position of the family? It has become clear that simply using the status of the husband as a measure of family stratification position is insufficient, but there is no agreed-upon method for assessing the contributions of husband's and wife's statuses to the stratification position of the family.

2. Does higher family stratification position result in children's valuing self-direction more highly? If so, what is the magnitude of the relationship between family stratification position and children's values?

-- Is it trivial, or does social stratification have a substantial relationship to children's values?

3. To what extent does the relationship between family stratification position and children's values result from stratification affecting parents' values, which in turn affect children's values? (It could be that social stratification affects children's values primarily through the influences of neighborhood and school; parents' values are not necessarily critical to this process.) If the process does involve parents' values, is it mothers' values, fathers' values, or both parents' values that is involved?

4. Assuming that social stratification does affect parents' values, what is the process? (Here the investigators lean on their earlier studies of U.S. and Polish men, which showed that occupational self-direction is of pivotal importance for explaining the impact of social stratification on values.)

5. What is the relationship between husbands' and wives' values? Do husbands' values affect their wives' values, do wives' values affect their husbands' values, neither, or both?

6. Is the relationship between parents' values and children's values unidirectional, from parents' values to children's values, or are the effects reciprocal, with parents' values both affecting and being affected by their children's values?

7. Insofar as parents' values do affect children's values, is it necessary for children's perceptions of their parents' values to be accurate?

8. Finally, are all the foregoing processes essentially similar for male and female children?

During this year, the investigators have made significant progress in answering the first five of these questions. In dealing with the first question -- the measurement of a family's social stratification position -- they explored several alternative methods of measurement, based on different conceptions of how husbands' and wives' statuses might contribute to the overall social position of the family. The most satisfactory model was produced by the method that made the simplest assumption: namely, that the position of the family in the social stratification system is best inferred from the covariation of husbands' and wives' educational levels, occupational statuses, and incomes. Technically, the model of family social stratification position developed for the U.S. is a third-order confirmatory factor-analytic model. The first-order components are multiple-indicator models of each partner's occupational status (based on several alternative indices of occupational status), together with less complex indices of educational attainment and job income. The second-order components combine husband's occupational status and income into a single concept, his occupational position; similarly for wives. Finally, the third-order component posits an underlying concept, the social stratification position of the family, inferred from the covariation of husband's educational level, husband's occupational position, wife's educational level, and wife's occupational position.

In the model for the U.S., the investigators find that husband's educational level and occupational position contribute somewhat more to the family's overall social stratification position than do wife's educational level and occupational status, but that the wife's statuses do make a substantial contribution to the family's overall position. [The standardized paths from family social stratification position to the four statuses -- which are analogous to "loadings" in an ordinary factor analysis -- are .84 for husband's education, .83 for husband's occupational position, .68 for wife's education, and .63 for wife's occupational position (limited to employed wives).] The model for Poland is constructed somewhat differently, mainly because data on income are available only in the form of combined family income -- but the results are similar. The only notable difference is that, for Poland, wife's education makes as much of a contribution to family social-stratification position as does husband's -- the standardized paths being .80 and .81, respectively. [The other components of the model are husband's occupational status, for which the standardized path is .73, wife's occupational status, .68, and family income, .52.] In both countries, then, there is very substantial covariation of husbands' and wives' statuses. It is meaningful to think in terms of the _family's_ position in the social stratification system. It is possible to index that position rigorously.

In further analysis (Question #2), the investigators found that family social stratification position, so indexed, is substantially correlated with _children's_ valuation of self-direction _versus_ conformity to external authority. For the United States, this correlation is .54; for Poland it is .31. (Here again it is not yet certain whether an apparent cross-national difference is real or is a statistical artifact. One can be certain, though, that in both countries the correlation is substantial.)

The real question, still, is not the magnitude of the correlation between family social stratification position and children's values, but the extent to which social stratification affects children's values through parents' values (Question #3 above). For the U.S., about two-thirds of the total effect of social stratification on children's values is indirect, through parents' values. Fathers play a somewhat larger role in this process than do mothers, both because fathers' values are somewhat more affected by social stratification than are mothers' values, and because fathers' values have a somewhat greater effect on children's values than do mothers' values. The key finding, though, is not the small difference in the importance of mothers' and fathers' roles, but that the effect of social stratification on children's values is mainly through parents' values, with fathers playing at least as large a part in this process as do mothers. The Polish model gives results that are similar in showing the effect of social stratification on children's values to be mainly -- in fact, entirely -- through parents' values. The Polish model differs from the U.S. model, though, in showing this process to occur more through mothers' than through fathers' values. (For the reasons stated above, it is not yet certain whether this is a true cross-national difference or a methodological artifact.) In any case, the crucial finding for Poland as for the U.S. is that parents' values play a key role in the effect of social stratification on children's values.

The next issue (Question #4 above) is to account for the effects of social stratification on parents' values. Here the investigators built on their prior analyses of U.S. and Polish men, testing anew the hypothesis that, to a very substantial effect, stratification affects parental values because parents' social stratification position affects their occupational self-direction and occupational self-direction in turn affects their values for children. As in past analyses, there are three stages to testing this hypothesis: First, the investigators developed models that establish the close relationship between social stratification and occupational self-direction. (These models show a strong unidirectional effect of education on occupational self-direction; they also show strong reciprocal effects between occupational position and occupational self-direction.) Second, the investigators found that the (unidirectional) effects of education are to some substantial degree, and of occupational position are almost entirely, indirect, through their effects on occupational self-direction, which in turn affects parents' values. Third, and crucially, they demonstrated in reciprocal-effects models that occupational self-direction does have an actual causal effect on parents' values. These analyses hardly come as a surprise, for all three models had been developed and tested in the investigators' prior analyses of these same data. These models do advance our knowledge, however, in two respects: The analyses are somewhat more precise than earlier analyses (being based on refined indices and full-information modelling); and the analyses have now been applied to working mothers in the U.S. and Poland, with results quite similar to those earlier found (and now reconfirmed) for U.S. and Polish fathers. The analyses, demonstrate, then, that for both fathers and mothers, in both the United States and Poland, social stratification affects parental values primarily through its effect on occupational self-direction, which in turn affects parents' values.

The investigators are currently working on the fifth question: the relationship between fathers' and mothers' values. The model is exceedingly complex, for it simultaneously includes the reciprocal effects of fathers' occupational self-direction with their own values, mothers' occupational self-direction with their own values, and fathers' values with mothers' values. Testing three pairs of reciprocal effects in one causal model is a difficult enterprise, one that, so far as we know, no one had previously succeeded in accomplishing. The investigators have solved such a model for the U.S., with these results: Mothers' values have a modest, albeit statistically significant, effect on fathers' values (the standardized path = .08); fathers' values have an even more modest, statistically nonsignificant, effect on mothers' values (path = .05). Essentially, each partner's values are derived from his or her own educational and occupational experiences, with little or no influence of either spouse's values on the other's. The investigators are now in process of developing a similar model for Poland. Analyses designed to answer Questions #6-8 are now underway. These constitute the agenda for the coming fiscal year.

EDUCATIONAL EXPERIENCE AND CHILDREN'S PSYCHOLOGICAL DEVELOPMENT

The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis, now nearing completion, is to examine the processes by which students' educational experiences, particularly the degree of self-direction they exercise in their educational endeavors, affect their psychological functioning. Data for this analysis were collected in the 1974 follow-up to the original 1964 survey, when the wife and one pre-selected child of each father in the sample was interviewed. The interview schedule for these "children" -- by then aged 13 to 25 -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions, designed to parallel those found to be useful for analyzing adults' occupational experience, focus on such dimensions of the educational experience as its substantive complexity and how closely it is supervised. The underlying hypothesis is that self-direction is important in young people's school experiences, just as in older people's job experiences. People who use initiative, thought, and independent judgment in their daily work, whether in school or paid employment, come to be more effective in their intellectual functioning, to have more self-directed orientations and values, and to be less distressed.

Empirical assessment of this hypothesis is hampered by the lack of longitudinal data for mothers and children. It is therefore not possible to statistically control earlier levels of psychological functioning in assessing the impact of educational self-direction on current psychological functioning, nor to statistically control earlier levels of educational self-direction in assessing the impact of psychological functioning on current educational self-direction. The investigators do, however, have one great advantage in this analysis: They have information about the psychological functioning of all of the students' fathers and most of their mothers. This means that, in assessing the impact of educational self-direction on the psychological functioning of students, they can statistically control parental psychological functioning. This makes it possible to take into account, to some substantial degree, family-experiential and genetic determinants of psychological functioning. A model of the reciprocal effects of educational self-direction and any facet of psychological functioning can thus include the corresponding facet of psychological functioning of each of the parents, as well as students' ages and grade levels, the extent to which school courses are compulsory or elective, and pertinent social characteristics of the students and their families.

Last year's report described a model for one important facet of psychological functioning -- ideational flexibility, the major aspect of cognitive functioning measured in this research. The main conclusion was that educational self-direction and ideational flexibility have substantial, approximately equal, reciprocal effects. The impact of educational self-direction on ideational flexibility results mainly from the substantive complexity of students' schoolwork -- its scope, difficulty, and challenge. During the current year, the investigators re-evaluated this model in light of an important question: the extent to which the findings are dependent on the assumptions made in estimating the model. Any reciprocal-effects model requires that certain assumptions be made -- usually, that some effects can only be indirect -- because otherwise there is insufficient information to disaggregate a single correlation into two reciprocal paths. This problem, known as the "identification problem", is even more thorny in cross-sectional analyses than in analyses using longitudinal data; in the absence of longitudinal information, there are less certain grounds for assuming that some effects can only be indirect. To determine the robustness of the results when these assumptions are relaxed, the investigators performed a series of tests, relaxing each of the assumptions in turn. The model proved to be quite robust, showing that, whatever assumptions are employed, educational self-direction always has a positive, statistically significant effect on ideational flexibility.

Last year's report also described preliminary versions of causal models of the relationship of educational self-direction with self-directedness of orientation and with distress. This year, these models were extensively refined and their robustness confirmed. Educational self-direction leads to a more self-directed orientation; this results almost entirely from the substantive complexity of schoolwork affecting self-directedness of orientation. The reciprocal effect, from self-directedness of orientation to educational self-direction, is positive but not statistically significant. The impact of educational self-direction on distress is, as expected, negative and substantial. Close supervision by teachers strongly increases distress, while substantively complex schoolwork decreases it. In the reciprocal causal direction, more distressed students are significantly less likely to exercise self-direction in their schoolwork. Thus, while educational self-direction affects both self-directedness of orientation and distress, the causal mechanisms are quite different for these two aspects of psychological functioning.

The investigators examined two other variables that they thought might be related to educational self-direction. First, they tried to develop a measurement model of leisure-time intellectuality, analogous to one previously developed for adults. The effort failed, because the intercorrelations among leisure-time activities are much weaker for students than for adults. This may be because much of students' leisure time is spent in school-related activities. In any case, the question of the impact of educational self-direction on leisure-time intellectuality cannot be resolved with these data. The second new effort, which proved successful, was to analyze the relationship between educational self-direction and valuation of self-direction versus conformity to external authority. The impact of educational self-direction on valuing self-direction is, as expected, substantial and positive. This results mainly from the positive effect of the substantive complexity of the schoolwork, but closeness of supervision by teachers also has a modest, statistically significant, negative effect on valuation of self-direction. In the reciprocal causal direction, self-directed values have a positive but statistically nonsignificant effect on the exercise of educational self-direction. The causal model for values, like those for other aspects of psychological functioning, was tested for robustness; the results proved to be highly robust, consistently showing a positive, statistically significant impact of educational self-direction on self-directed values.

The investigators are now attempting to build a larger causal model encompassing the reciprocal interrelationships of educational self-direction, intellectual functioning, self-directedness of orientation, and distress. Such a model, if successful, will show not only the independent effects of each variable (with all the others statistically controlled), but also the processes by which these effects occur. In particular, the model is designed to show whether educational self-direction directly affects all three aspects of psychological functioning, or whether, for example, educational self-direction affects ideational flexibility and distress partly through its effect on self-directedness of orientation, as is the case for adults' occupational self-direction. Successfully developing the expanded model, though a complex task, should greatly expand our knowledge of the relationships between educational experience and psychological functioning.

Whatever the larger model shows, the analyses already completed have demonstrated that the "quality" of the educational experience does make a difference for students' psychological development. The substantive complexity of schoolwork is primary in importance; more substantively complex schoolwork leads to more effective intellectual functioning, to a more self-directed orientation, and to greater valuation of self-direction. Closeness of supervision by teachers affects neither intellectual functioning nor self-directedness of orientation, but does have a modest, statistically significant effect on values. Moreover, closeness of supervision, rather than the substantive complexity of schoolwork, is what affects distress: the more closely supervised, the more distressed is the student. These findings parallel what has previously been found for adults in the workplace and thus extend our knowledge of how work and personality affect one another in different institutional settings and at different stages of the life-course. In particular, they show that not only for adults, whose work is paid employment, but also for children and young adults, whose work is schooling, the experience of self-direction in work is important for psychological functioning.

INTRA-FAMILY DYNAMICS AND CHILDREN'S PERSONALITY DEVELOPMENT

This year Carmi Schooler began working on an investigation of the ways that reported child-rearing practices and family relationships affect children's psychological functioning. In its initial stages, this analysis involved substantial recoding of interview responses. A major reorganization of the data files was also required, so that the data from fathers', mothers', and children's interviews could be analyzed together. Once these tasks were accomplished, confirmatory factor analysis was used to develop measures of the many aspects of parent-child relationships covered by the interviews. Indices were developed of such aspects of parents' relationships with their children as strictness, warmth, and degree of dominance over the child, as well as such measures of the children's responses to their parents as the degree they feel free to talk things over with each parent and the likelihood they will turn to each parent when troubled. The data have now been arranged in the form of a correlation matrix, necessary for the next step in the investigation -- the use of linear structural-equation analysis to explore the ways that parental practices and parent-child relationships affect the psychological functioning and values of children.

EFFECTS OF HUSBAND'S AND WIFE'S SOCIAL STATUS ON PSYCHOLOGICAL FUNCTIONING

Carrie Schoenbach has used the data of the U.S occupations study to test the validity of a commonly-held assumption in social-psychological research -- that the social stratification position of a family is defined by the status of the husband (using such measures as his education, income, and occupational status). It becomes critical to examine this assumption when we ask about husband's and wife's psychological functioning in families where both spouses are employed because, as research in this Laboratory has established, social stratification position matters for psychological functioning primarily because of the impact of occupational conditions, which are systematically associated with social stratification position.

Schoenbach's analysis asks whether the personalities of employed wives are, in fact, related more to their husbands' social stratification positions or to the women's own positions. The facets of personality examined are self-directedness of orientation, distress, and intellectual flexibility. The model uses linear structural-equations analysis to estimate the effects of each partner's education, occupational status, and income on both own and spouse's personality. The effects of status on personality are examined separately for each aspect of personality. The consistent finding is that, for wives as well as for husbands, it is one's own social stratification position that is of predominant importance for personality. Insofar as husband's status does affect wife's personality, it appears to be primarily through his status affecting his own personality, which in turn affects his wife's personality.

THE POLISH REPLICATION

The main purpose of the Polish replication has been to see whether the interrelationship of social stratification, job conditions, and psychological functioning is similar in socialist and capitalist societies. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed the development of methods designed to assure cross-national comparability of indices and the analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? As reviewed in detail in earlier Annual Reports, the answers to both questions are positive with respect to values and social orientations, but not with respect to self-conception.

Further comparative analysis of the Polish and U.S. data has focused on social stratification and the intergenerational transmission of values (discussed above) and three further questions.

(1) The first question is whether, in both countries, the relationships between occupational self-direction and intellective process are similar for younger, middle-aged, and older workers. Recent work in the developmental and social psychologies of aging suggests that learning, particularly as represented in "crystallized intelligence," continues throughout the life-span. In principle, since "transfer of learning" is an essential characteristic of the learning process, not only initial learning but also the generalization of what has been learned should continue as workers grow older. It is nevertheless possible that learning-generalization does not occur at the same rate or to the same extent at all ages and all stages of career and life-course. The process may be especially pronounced in younger workers, at early stages of their occupational careers, and before they are preoccupied with family responsibilities, but may diminish as workers grow older, advance in their careers, and have changing family responsibilities. It is also possible that either learning or generalization diminishes as workers grow older, simply because of biological decrements. To see whether learning and the generalization of learning continue unabated throughout adult life requires an analysis of how job conditions affect the psychological functioning of workers at different ages, or stages of career, or stages of life-course. Joanne Miller, Kazimierz M. Slomczynski, and Melvin Kohn have attempted such an analysis. They believe that this analysis is the first systematic empirical effort to see whether learning-generalization continues to be

responsive to the social-environmental conditions of natural settings as workers grow older.

The analysis is cross-national: It examines the continuity of learning-generalization in two countries, Poland and the United States. As in any analysis of the relationship between social structure and personality, cross-national comparative analysis has the utility of ascertaining whether the findings in any one country are specific to the culture and to the economic and political system of that country. An analysis of people of differing ages offers the further advantage that even the "same" cohorts have had different experiences in different countries. When the two countries are Poland and the United States, we also have the opportunity of seeing whether the findings are consistent for a socialist and a capitalist society.

For both Poland and the United States, the investigators did separate analyses of job conditions and intellective process for younger, middle-aged, and older men. For each country, their analysis asked: Is the relationship of job to intellective process consistent for all three age-groups? Since a rigorous test of between-group similarities should allow the possibility that men of differing age-groups vary in how their answers to the interview questions relate to the underlying concepts one wishes to measure, the analysis began with the construction of separate measurement models of all major concepts for each age-group. The measurement models proved to be very similar for all three age-groups in both countries.

The main conclusion of these analyses is that job conditions affect intellective process in older men just as much as in younger men. In particular, job conditions facilitative of the exercise of self-direction in work continue to enhance ideational flexibility and an open-minded, non-authoritarian orientation, even in the "oldest" segment of the workforce, that is, among men aged 46 to 65 years. These findings emerge both in cross-sectional and in longitudinal analyses for the United States. For Poland, where only cross-sectional analyses are possible, the analyses indicate that the findings apply as well to that country. Learning and generalization from the experiences of work occur regardless of age, and -- if one may extrapolate -- regardless of stages of career and life course.

Moreover, the investigators have discovered notable continuity not only of the effects of job conditions on intellective process, but also of the effects of intellective process on job conditions -- a continuity of reciprocal process into later stages of career and later stages of life course. Just as men continue to learn from their jobs and to generalize those lessons to outside-of-job reality, so too do men continue well into their careers to select and to mold their jobs to fit their intellective proclivities.

Finally, one can consider the findings of this analysis from the perspective of what they tell us about the generality of the effects of occupational self-direction on psychological functioning. The present analysis shows that occupational self-direction, particularly the substantive complexity of work, has a decided effect on ideational flexibility in Poland -- a new and important piece of evidence. More generally, the findings tell us that the relationship between occupational self-direction and intellective process holds for six groups of men who have had decidedly different generational and historical experiences. The generality of the relationship is thus extended not only with respect to age, but also with respect to diversity of experience.

(2) Krystyna Janicka (Polish Academy of Sciences), Grazyna Kacprowicz (University of Warsaw), and Kazimierz Slomczynski continued their analysis of the Polish data, evaluating the reliability of the measurement model of occupational self-direction. They found the multiple correlation between the overall index of the substantive complexity of work with the independently coded ratings of the complexity of work with things, with data, and with people given in the Polish version of the *Dictionary of Occupational Titles* to be 0.81, almost exactly the same as Kohn and Schooler had found it to be for the U.S. They then went on to assess the validity of the index by comparing it to evaluations of job complexity based on much more detailed descriptive information about job content. To do this, they re-interviewed subsamples of their national sample in two industrial cities, Lodz and Wroclaw. These interviews provided more detailed information about job content than it would be possible to obtain in any broader survey. Using a number of alternative coding schemes, they found high correlations (in the .90's) between indices of the substantive complexity of work based on brief and on detailed descriptions of the job. These investigators found also that direct (subjective) questions about the complexity of work provide only biased appraisals of the substantive complexity of the work and cannot substitute for descriptive questions about the actual content of the job.

(3) Slomczynski carried out an analysis of the effects of status inconsistency on psychological functioning, using the Polish data. He used formal education, occupational prestige, and job income as indices of social stratification. His major assumption is that status inconsistency should be separated from status *per se*, by methods of measurement that assure the orthogonality of the constructs. Accordingly, status is conceptualized as the weighted sum of education, occupational prestige, and income, while status inconsistency is conceptualized as the weighted difference among all three pairs of indices of stratification. He adapted principal-component analysis to obtain an orthogonal solution for both constructs. Since status is, by this method of construction, the first component, its effects on psychological functioning must be greater than those of status inconsistency. Still, the question remains whether the psychological effects of status inconsistency are statistically significant and non-trivial in magnitude.

Significant effects of status inconsistency were found in the domain of intellective process, measured by ideational flexibility and authoritarian conservatism, as well as in the domain of social orientation, in particular, trustfulness and personally responsible standards of morality. Ideational flexibility linearly decreases while authoritarian conservatism increases with the extent to which education and prestige are disproportionately high relative to income. Trustfulness and personal standards of morality decrease with status inconsistency, especially if income is disproportionately low. Status inconsistency adds substantially to the overall explanation of the variability of psychological functioning. For example, status alone explains less than half of the variance of ideational flexibility (47 per cent); together with status inconsistency, it explains 54 per cent of the variance. The effects of status inconsistency remain statistically significant when respondent's age and his father's occupation -- two variables that are closely related to psychological functioning -- are controlled. The effects of status inconsistency on trustfulness and personally responsible standards of morality even increase slightly in magnitude when the respondent's age and his father's occupation are statistically controlled.

Slomczynski intends now to replicate the analysis with our U.S. data.

THE JAPANESE REPLICATION

Another major replication of the Kohn-Schooler occupations study has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. Data-collection took place during the summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed, using a questionnaire that asked about job conditions and aspects of psychological functioning in ways similar to those of the 1964 U.S. study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work collaboratively with Carmi Schooler. As reported in previous Annual Reports, Naoi and Schooler developed confirmatory factor-analytic measurement models of occupational self-direction, intellectual flexibility, and several facets of self-conception and social orientation. These models proved to be generally similar to those that had previously been developed for U.S. and Polish men. Causal analyses of these data generally confirmed the U.S. and Polish findings.

This year, Schooler and Tominaga began an investigation of the social structural determinants of self-directed values in Japan. Using linear structural-equations analysis, they developed a measure of paternal valuation of self-direction *versus* conformity to external authority that corresponds to the measures developed earlier for U.S. and Polish parents. It proved more difficult to develop an appropriate measure of Japanese men's valuation of self-direction *for themselves*. Such a measure was eventually developed, but it differs somewhat from the U.S. measure of self-values, in that the "conformity" pole of the dimension includes getting along with peers, while in the U.S., it is restricted to conformity to external *authority*.

Schooler and Tominaga next examined the relative effects on the valuation of self-direction for one's children and for one's self of several social structural characteristics: occupational status, economic status, educational status, social status of family of origin, age, urbanness, and employment in a relatively traditional *versus* a more modern sector of the economy. For self-values, occupational and educational status proved by far the most potent determinants, although being younger and working in a non-traditional setting also have statistically significant effects. The powerful effects of occupational and educational status on self-directed values for one's self replicate U.S. findings.

Social-stratification position as evidenced by occupational and educational status is also among the most important determinants of self-directedness of parental values. Social-structural variables other than social stratification, though, have relatively greater effects for parental values than for self-values. Thus, age of parent has a stronger effect on parental values than does educational and occupational status, younger fathers having more distinctly self-directed parental values than older fathers. An urban background and a relatively non-traditional work setting also have greater effects on self-directedness of parental- than of self-values. Despite the differences in the relative importance of particular social structural characteristics for parental- and for self-values, the results are congruent with earlier U.S. and Japanese findings about the ways that complex, multifaceted environments affect psychological functioning.

During this year, Schooler also began analyses to explore the possibility that the sector of the economy in which an individual works may affect his psychological functioning or may affect the ways that job conditions affect psychological functioning. In the past decade or so, there has been an increased interest in the economic organization of industry, resulting in an extensive literature about a "dual economy." Although there are shades of difference among them, most dual economy theories distinguish between a primary sector -- composed of industries marked by high levels of oligopolistic control, large economic scale (including size of work force and assets), relatively large internal labor markets, and relatively secure workers -- and a secondary sector, made up of industries in which firms tend to be smaller, both in assets and size, and less well coordinated with each other, and in which workers are generally less well off and less secure. If this distinction is valid, the psychological functioning of individuals may well be affected by the social atmosphere of the sectors in which they work. Thus, working in the secondary sector, where jobs are generally less secure and workers are more subject to arbitrary decisions by superiors, may affect the level of the individual's distress, self-directedness of orientation and intellectual flexibility. Moreover, job conditions may have different psychological effects on workers in the primary and secondary sectors of the economy.

Some empirical tests have been made of the dual economy conception. The most comprehensive, carried out by Tolbert, Horan, and Beck, used principal component analysis of company characteristics in a number of industries (e.g., assets, profit, number of workers, average weekly wage) to see whether these characteristics covary in the ways that dual economy theory predicts. These investigators extracted a single factor that they see as differentiating industries in the primary and secondary sectors. Schooler has recoded pertinent data from our occupations study about the organizations for which the respondents worked, to take advantage of the coding scheme Tolbert et al. have provided. In addition, in collaboration with Ronald Schoenberg, he is using confirmatory factor analysis to reanalyze the original data on which Tolbert et al.'s analysis was based. Initial analyses suggest that the covariance of company characteristics is best explained by two factors, one factor essentially measuring the company's success in attaining its corporate goals, the second the success of workers in achieving good wages and job security. If further analyses prove that such a factorial solution is appropriate, the psychological effects of these two characteristics of industries will be explored.

WORK EXPERIENCES AND THE DEINSTITUTIONALIZED MENTALLY ILL

One year ago, Elliot Liebow, on detail to the Laboratory from the Extramural Program, began an exploratory, participant-observer study of the relationship between work experience and recovery from mental illness. The goal of this exploratory research is not to test hypotheses but rather to grasp, so far as possible, the dynamics of the interaction between work experiences and recovery from mental illness.

In this first year, data collection has been based mainly on direct observation and personal interaction with men and women coming out of Springfield State Hospital and following them through their reentry into their home communities, focussing particularly on the role of work experiences in this process. The study population is drawn mainly fom half-way houses, psychosocial and vocational programs, soup kitchens, and emergency shelters in Montgomery County, Md.

The data collection phase is expected to end in September of this year. For the remaining five months, the project will continue to focus sharply on the individual work experiences over time of deinstitutionalized persons, but a greater effort will be made to place these experiences in a larger social and mental health system context. The social context will be further developed through direct personal contacts, formal and informal, with employers, supervisors, co-workers, friends, parents, and other relatives. The mental health system context will be constructed from interviews with hospital administrators in charge of vocational programs and discharge planning, after-care teams, case managers, and Federal, state, and county representatives of vocational rehabilitation programs, and other relevant social and rehabilitative mental health services.

METHODOLOGICAL INQUIRY

A large part of the research conducted by members of this Laboratory depends on a data analysis technique and associated computer program developed and maintained by Ronald Schoenberg. The program is called MILS, which stands for Multiple Indicator Linear Structural analysis. Many revisions and additions were made to the computer program during the year to meet changing demands and to keep the methods used in the Laboratory as current as possible. For example, improved methods were developed and added to the program to aid in the evaluation of the fit of the models. A Generalized Fit Index is now calculated that is not influenced by sample size, and summary measures of explained variance for the structural and measurement models are now computed.

Significant progress has also been made in the analysis of the interaction effects of latent variables. Important problems in this method of analysis have been solved during the year. The greatest flexibility in this kind of analysis will be achieved through the use of factor scores constructed from indicators of the concepts in the model. Interaction effects are studied by multiplying the factor scores together and including these product variables in the model. Factor scores are themselves fallible measures of the true scores and therefore a method for correcting the fallibility had to be developed. In addition, a method was developed to calculate the standard errors of the coefficients of the interaction terms, so that correct inferences might be drawn with regard to their statistical significance.

Annual Report of Laboratory of Cell Biology
National Institute of Mental Health
Michael J. Brownstein, M.D., Ph.D., Chief
October 1, 1983 - September 30, 1984

INTRODUCTION

The members of the Laboratory of Cell Biology (LCB) are currently in the midst of moving from Building 10 to Building 36. In addition to the two senior investigators who were members of the Laboratory one year ago (Drs. Brownstein and Zatz), a third senior investigator has recently transferred to the LCB, Dr. Axelrod. Drs. Hirata, Pruss, and Eiden continue to function as Unit Chiefs. They have been joined by other intermediate-level investigators: Drs. Bonner, Hook, Moskal, Mezey, and Chesselet. Dr. Palkovits continues to function as half-time chief of the anatomy group. He will be in Bethesda from June 1984 to January 1985 along with his wife, a superb EM technician who plans to work in the lab and whose talents will be much appreciated.

Several people plan to come to the LCB by autumn of this year including D. Marshak, W.S. Young, T. Kilduff (all P.R.A.T. fellows), A. Rokaeus, D. Kligman and T. Zoeller. Each of the above will bring new skills and new perspectives to the lab and we look forward to their arrival.

Work in the Laboratory continues to be broad based, ranging from neuroanatomy to molecular biology. Neuroanatomical studies have been conducted by Drs. Palkovits, Mezey, Kiss and Chesselet. These workers have used light level immunocytochemistry (with and without retrograde tract tracing techniques) to study diverse neuronal pathways in the CNS. Furthermore, they have examined the effects of physiological and pharmacological manipulations on patterns of peptide staining in hypothalamic neurons and have gained new and important insights into the role of these neurons in neuroendocrine control.

In addition to light microscopic investigations, EM work will soon get under way. The EM will be valuable for a broad range of cell biological studies--studies stimulated by the availability of antibodies against molecules of biological interest (e.g., carboxypeptidase "E," lipomodulin, "docking protein," etc.).

Much of our work continues to involve purification and characterization of molecules. This should allow us to approach the goal of reducing biological phenomena to a molecular level. Our ability to sequence peptides and proteins at the subnanomole level and to synthesize oligonucleotides and peptides should contribute significantly to the achievement of the above goal.

SPECIFIC PROJECTS

Drs. Siegel, Brownstein, and Okayama are attempting to use expression cloning in mammalian cell lines to characterize neurotransmitter receptor mRNA's. This long term project should result in a better understanding of the structure and function of receptors.

Drs. Brownstein, Gainer and Loh are continuing to investigate mechanisms of peptide precursor processing. Work in progress indicates that processing is more complex than heretofore suspected. In addition to a trypsin-like endopeptidase that recognizes pairs of bases and a carboxypeptidase B-like enzyme, a second endopeptidase that cleaves after single bases and an aminopeptidase that trims off basic residues from the N-terminus of peptides may be involved in processing.

Dr. Hook has studied one of the above enzymes in detail: the carboxypeptidase B-like enzyme named carboxypeptidase E to distinguish it from the former. She has developed specific antibodies against it (with Drs. Fricker and Snyder) and in collaboration with Drs. Mezey, Pruss, and Siegel, has used these for immunocytochemistry. She has also developed a RIA for the enzyme and should now be able to study the regulation of its synthesis. She has developed an assay for the trypsin-like enzyme that cleaves between or after pairs of bases and has begun to isolate and characterize it.

Drs. Zamir, Palkovits, Mezey, Weber, and Brownstein have shown that destruction of fibers traveling from the basal ganglia to the substantia nigra results in a marked decline in dynorphin-related but not met-enkephalin-related peptides. The lesions also cause leu-enkephalin levels in the substantia nigra to fall precipitously. Thus, it seems that leu-enkephalin can be liberated from the dynorphin/neuroendorophin precursor.

Drs. Namboodiri and Brownstein have studied the distribution of an enzyme that acetylates aspartic (but not glutamic) acid. This enzyme may be involved in the biosynthesis of N-acetyl Asp Glu.

Drs. Mezey, Reisine, Palkovits, and Axelrod have continued to study the role of peripheral catecholamines in regulating pituitary ACTH secretion. They have shown that catecholamines mediate the outpouring of ACTH that is seen after insulin administration.

Drs. Mezey, Kiss, Reisine, and Axelrod have shown that the CRF-producing neurons in the paraventricular nucleus receive a dense adrenergic innervation and that blockade of adrenaline synthesis with PNMT inhibitors causes a significant increase in CRF production.

Dr. Kiss and Dr. Mezey have found that the same set of cells that make CRF can also synthesize vasopressin and cholecystokinin. This is especially apparent in adrenalectomized animals. Together with Dr. Reisine they have shown that vasopressin plus CCK are as effective as CRF in releasing ACTH from cultured pituitary cells.

Dr. Moskal has prepared and identified a monoclonal antibody that appears to bind to a molecule(s) involved in modulating a specific glycoprotein sialyltransferase since its removal from solubilized Golgi preparations by immunoprecipitation results in a marked elevation in this enzyme activity. He has also found a four-fold increase in the specific glycoprotein sialyltransferase activity mentioned above when NG 108-15 cells are differentiated with dibutyrl-cyclic AMP. Retinoic acid, on the other hand, elevates another sialyltransferase--one involved in the biosynthesis of GM3 ganglioside. Finally, when NG 108-15 cells are differentiated with dibutyrl-cyclic AMP and cocultured with primary muscle cultures the activity of the galactosyltransferase involved in production of lactoneotetraosylceramide was increased. Dr. Moskal is attempting to identify the factors that cause these changes in enzyme activity.

Dr. Moskal has raised monoclonal antibodies to five day postnatal rat dentate gyrus granule cells. The antibodies recognize cell surface antigens and have proven useful for fluorescence activated cell sorting. Thus normal and abnormal (reeler mutant) granule cells can be separated and their surface molecules compared.

Dr. Russell has studied two transport processes in neurosecretory vesicles: 1) the transport of protons and 2) the transport of electrons. The first of these is mediated by an ATP-dependent system and functions to maintain the intravesicular pH between 5.2 and 5.7. The low intragranular pH may promote condensation of peptide precursors and aggregation of products. It may also be optimal for (or regulate) the activity of processing enzymes. The electron transfer mediator appears to be a cytochrome (b_{561}) in the granule wall. Transport of electrons to the inside of the granules is essential for the activity of the peptide amidating enzyme.

Dr. Pruss has raised a monoclonal antibody against bovine cytochrome b_{561}, has used it to examine the tissue distribution of the cytochrome, and plans to examine the regulation of its production.

Drs. Pruss, Siegel, and Eiden have studied the effects of drugs on peptide levels in cultured chromaffin cells. VIP levels increase markedly in the cultured cells; the mechanism of this effect is being sought.

Drs. Eiden, Affolter, Giraud, Hotchkiss, Siegel, and Pruss have investigated the effects of nicotine, forskolin, potassium, and reserpine on enkephalin and related peptides and on messenger RNA coding for proenkephalin ($mRNA^{enk}$). Nicotine and forskolin increase intracellular enkephalin and $mRNA^{enk}$ levels, nicotine rapidly and forskolin more slowly. Depolarizing agents (e.g., K^+) also increase enkephalin and $mRNA^{enk}$ at the same time that they provoke release. It appears that stimulation of enkephalin biosynthesis occurs via enhanced enkephalin gene transcription following an increase in intracellular cyclic AMP, and may depend on calcium influx as well. Bovine enkephalin gene has been isolated and studies are in progress of transcriptional regulation of $mRNA^{enk}$ production.

Dr. Eiden, Ms. Iacangelo, and Ms. Hsu have established a RIA for chromogranin A and have shown that this molecule constitutes about 10 percent of the total protein in chromaffin cells. They have prepared a bovine adrenal medulla cDNA library and are screening this library for chromagranin A clones.

Drs. Bannon, Giraud, Affolter, and Eiden have developed a method for quantitating $mRNA^{enk}$ in discrete brain regions and hope to use it to gauge neuropeptide turnover. They have shown that haloperidol increases $mRNA^{enk}$ within several hours (at a time when enkephalin concentrations are unchanged).

Dr. Giraud has used pulse-chase methodology to show that drugs which increase or mimic cyclic AMP increase enkephalin precursor and enkephalin pentapeptide production by adrenal medullary cells. Cholinergic agents have the same effect while reserpine acts posttranslationally to stimulate the processing of enkephalin precursor as opposed to its synthesis.

Drs. Siegel and Kiss have made significant progress towards developing a reliable and well validated method for localizing (and quantitating) mRNA by in situ hybridization cytochemistry. They have found that most cultured chromaffin cells have $mRNA^{enk}$ and that following growth in depolarizing medium message levels increase. Preliminary attempts to combine in situ hybridization and immunocytochemistry have been encouraging.

Dr. Antoni has found that the anterior pituitary has a single class of high affinity binding sites for arginine vasopressin and that these sites are different from those previously described in rat tissues. The adrenal medulla also has vasopressin binding sites identical to those in bovine liver membranes. The role of vasopressin in the adrenal medulla is under investigation.

Drs. Moskal and Zatz have transformed pineal cells from hatchling chicks using a temperature-sensitive virus. They will screen these cells for those which retain the properties of circadian pacemakers. Establishment of a clonal cell line with circadian properties would permit biochemical analysis of the mechanisms underlying circadian rhythms.

Drs. Zatz and O'Brien have discovered and solubilized the enzyme which transfers long chain fatty acids to rhodopsin from acyl coenzyme A. This enzyme mediates a new class of posttranslational modification of membrane proteins, including receptors.

Dr. Zatz has developed a procedure for analysis of inositide metabolism in small amounts of tissue. He is investigating the relationships between alpha-adrenergic stimulation, inositide metabolism, melatonin production, and lithium in the rat pineal. Lithium has the unusual effect, in this system, of potentiating physiologic potentiation, without either stimulating or potentiating on its own.

Drs. Zatz and Reisine have found that lithium stimulates ACTH secretion by anterior pituitary cells in culture and by pituitary tumor (AtT-20) cells. Inositide metabolism is concomitantly affected. Also, lithium desensitizes the cells to subsequent stimulation by lithium (or by phorbol esters) but not by other agonists.

Dr. Hirata and his colleagues have succeeded in purifying lipomodulin and in obtaining a partial amino acid sequence for it. Based on this sequence they hope to construct oligonucleotide probes and to detect DNA complementary to lipomodulin mRNA.

Drs. Hattori, Hirata, and Notsu have shown that lipomodulin can alter the phenotype of neuroblastoma (NH15CA2) cells from cholinergic to adrenergic.

Drs. Kunos, Hirata, and Rouben have demonstrated that lipomodulin can affect the adrenergic receptor subtypes on rat hepatocytes.

Annual Report of the Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

October 1, 1983 - September 30, 1984

Paul D. MacLean, M.D., Chief

Both this year and next promise to be remembered for two red-letter events in the field of paleoanthropology. This last April an unparalleled exhibit entitled "Ancestors: Four Million Years of Humanity" opened at the American Museum of Natural History. It brought together 50 of the most notable finds of fossils revealing the evolution of humankind. Some viewers would contend that no museum piece of any kind generates the awe and wonderment experienced upon seeing the beautifully sculptured brow and cheekbones of the "Taung child" of South Africa. Next February, in commemoration of Raymond Arthur Dart's announcement of the discovery of the Taung skull 60 years ago, there will be a Diamond Jubilee International Meeting at the University of Witwatersrand, Johannesburg. Dart identified the skull as that of a new species to which he gave the name Australopithecus africanus. Breccia found at another site raised the question about the possible use of fire by the australopithecines, a matter that has become of current interest because of circumstantial evidence elsewhere in Africa of hominid control of fire "dating to 1.0-1.5 million years ago."

These introductory considerations invite a few comments on the developing field of Evolutionary Psychiatry and how the work of the Laboratory relates to it, citing, for example, one study on the separation call that raises the question about the use of fire by human antecedents. What is meant by evolutionary psychiatry? The word psychiatry itself means "mind healing." Evolutionary psychiatry is concerned with obtaining salutary insights derived from a better understanding of how the brain has evolved and functions. Starting with the subjective self as its province (its territory), the domain of evolutionary psychiatry encompasses both the micro- and macroscopic aspects of all the underlying phenomena as they seem to unfold in past-present-future and relate to the cosmos.

In evolving to its great size the human forebrain has retained the anatomical organization and chemoarchitectonics of three neural assemblies that reflect an ancestral relationship to reptiles, early mammals, and late mammals. Apropos of evolutionary psychiatry such considerations provide the rationale for conducting comparative neurobehavioral studies in an attempt to identify commonalities and differences in the way animals derive information and behave.

In the evolutionary transition from reptiles to mammals three key behavioral developments were nursing in conjunction with maternal care; audio-vocal communication for maintaining maternal-offspring contact; and play. Comparative neurobehavioral work in our Laboratory is continuing to involve experiments suggested by evidence that the behavioral triad in question is represented in the thalamocingulate division of the limbic system. Significantly, there appears to be no counterpart of this subdivision in the reptilian brain. The present focus is on the so-called separation (isolation) call which is perhaps the most primitive and basic mammalian vocalization, serving originally to maintain maternal-offspring contact. The results of this year's experiments on squirrel monkeys provide a further delimitation of the cortical areas involved in the spontaneous production of the isolation call. The critical area appears to include the paragenual and preseptal cingulate cortex and perhaps also the immediately adjacent medial frontal neocortex.

In regard to the separation cry, it is physiologically of special interest that human beings are the only creatures known to shed tears with crying. Human beings and their antecedents are the only creatures known to have used fire. Is it possible that in the course of millions of years there resulted an association between smoke and tears and activities surrounding fire, including ceremonies involved in disposing of the dead? This line of thought also calls to mind the unexplained tearing experienced upon witnessing an altruistic act. The prefrontal cortex, which is clinically recognized to be involved in the generation of altruistic sentiments, is anatomically linked to the thalamocingulate division shown by animal experimentation to be implicated in the separation call, parental behavior, and play. Is it possible that tearing upon seeing an altruistic act reflects a high order generalization owing basically to a close association of neural mechanisms involved in crying and in parental rescue? Apropos of evolutionary psychiatry, potentially salutary insights might be expected from knowledge of neural mechanisms involved in separation reactions--particularly as applied to various conditions of depression and excessive grief, as well as forms of mental illness, drug abuse, and alcholism sometimes ascribed to traumatic maternal separation during infancy or childhood.

In prosematic (non-verbal) communication terrestrial animals rely principally on four main forms of behavior referred to in ethological literature as displays. As originally shown in this Laboratory, the medial segment of the globus pallidus and its projecting pathways play an essential role in the evocation and performance of species-typical displays. The pallidum is part of the striatal complex which forms a basic part of the forebrain in reptiles, birds, and mammals. This year's work has provided further information about brain structures involved in the vocal and autonomic components of a species-typical display of one variety of squirrel monkeys. Other work of the Laboratory has continued to deal with the function of the striatal complex at the molecular level, focusing particularly on the role of iron which occurs in greater concentration in the pallidum than in any other structure of the forebrain. Iron is known to be involved not only in oxidative functions, but also in the synthesis, degradation, and binding of neurotransmitters. Further work on one project has indicated that the presence of iron is related to the metabolism of gamma-aminobutyric acid, a major inhibiting neurotransmitter. Another study has dealt with the localization of transferrin, an iron-binding serum protein that delivers iron to cells by binding to specific receptors on cell surfaces. It was found that the localization of transferrin receptors does not coincide with brain areas rich in iron. In the case of the striatal complex transferrin receptors are located in structures that project to the iron-rich pallidum.

Experiments conducted in the Unit for Research on Behavioral Systems (URBS) have made it possible for the first time to tabulate successive behavioral changes on a day-to-day basis over a prolonged period of time and to assess how brain damage or ecological factors (e.g., crowding) affect specified behaviors of individual animals living in large groups in complex environments. This accomplishment has been made possible by the subcutaneous implantation in rats of passive resonators that signal to a computer each time an animal passes through a portal, thereby identifying its locus, as well as the time and direction of its movement. Thus it has been possible, for example, to piece together how the presence of a female in estrus alters the hour-by-hour activities of all members of a colony.

In one URBS study, the behavior of micrencephalic rats and of control animals living in different sized colonies was compared. The chemically induced micrencephaly was characterized by severe alteration of the limbic and neocortex in the posterior half of the hemispheres. Compared with the controls, the micrencephalic rats required a longer time to learn to cope with a complex environment. Under relatively crowded conditions, the micrencephalic rats also proved to be more pugnacious and demonstrated poorer parental behavior. In another URBS study on normal rats, an analysis is being conducted to assess changes in vocal communication as animals learn to engage in cooperative behavior. Finally, in an investigation of the effects of overcrowding on mice living in a structured environment, analysis has revealed the following notable changes: As population density increases, there is a decrease in residential stability. At the same time there is an increase in aggressivity and a decrease in successful parentage.

Finally, it is gratifying to note a further development resulting from the cooperation of the Institute of Child Health and Development and our Institute. Since the establishment last year by Child Health of the Laboratory of Comparative Ethology under the direction of Stephen Suomi, the outdoor habitat for rhesus monkeys that encompasses Hazel's Pond is already in operation, with its opening having been officially celebrated on June 7.

Annual Report of the Laboratory of Cerebral Metabolism
National Institute of Mental Health

Louis Sokoloff, M.D., Chief

October 1, 1983 through September 30, 1984

The Laboratory of Cerebral Metabolism continues to consist of two Sections with independent research programs that have in common an orientation toward biochemistry of the nervous system. Although their programs are distinctly different, the two Sections do share equipment, facilities, and methodology, particularly with respect to analytical biochemical procedures. Both Sections have, for the most part, continued to emphasize research on previously initiated projects, but have been handicapped by relocation of part of their resources and the reconstruction that has been in progress for several months and is still not completed. Despite these physical encumbrances significant progress has been made in all areas of research.

Section on Developmental Neurochemistry

Louis Sokoloff, M.D., Chief

The research of this Section has been oriented mainly in two directions: the completion of previously initiated projects to clear the facilities and resources for new initiatives, and the defense of the deoxyglucose method (developed in this Section) against what are considered to be unwarranted and illegitimate, politically motivated attacks.

Previously initiated projects that have been completed and published or are in the process of being published have been in the area of neuropharmacological applications of the deoxyglucose method. Some of these have been studies of the effects of caffeine and cholecystokinin-octopeptide on local cerebral metabolism. Both agents were found to alter local rates of glucose utilization in patterns that suggest some interaction of these agents and dopaminergic systems. Both the caffeine and the cholecystokinin studies have been published by Nehlig et al. and Lucignani et al., respectively.

Namba, Porrino, and Nehlig have completed their studies on the changes in local cerebral glucose utilization with the stages of the estrus cycle on female rats and on the effects of high doses of estrogens. These studies have been published or are in the process of being prepared for publication. They show that glucose utilization varies in specific regions of the brain, mainly hypothalamic, with the stage of the estrus cycle and that high doses of estrogen cause widespread increases in glucose utilization throughout the brain.

Porrino and Esposito have been carrying out and have published some aspects of some truly pioneering studies. They have employed the deoxyglucose method to help identify the central nervous substrates of the phenomenon of rewarding self-stimulation. This phenomenon is believed to represent a model for motivational or hedonistic behavior. They have found in rats that self-stimulation via electrodes placed in the ventral tegmental area or in the zona compacta of the substantia nigra produces a distribution of changes in local cerebral glucose utilization distinctly different from that seen in animals given comparable electrical stimulation by the investigators. Of particular interest is the finding of bilateral changes in the self-stimulating animals in the amygdala, nucleus accumbens, and other regions of the prelimbic system, changes not seen in the control animals. These results indicate that these structures may be involved in motivational behavior. Portions of these studies have already been published and have aroused considerable recognition and acclaim.

Smith has been continuing her studies to refine the newly developed method for measurement of local rates of cerebral protein synthesis and to apply it to problems of neurobiological interest. She has completed her studies on the effects of axotomy on protein synthesis during regeneration in the hypoglossal pathway. She has found activation of protein synthesis and glucose utilization in the hypoglossal nucleus to occur soon after hypoglossal nerve section and to continue until regeneration of the nerve and maximum return of function has occurred. A report of these studies has been accepted for publication and is now in press.

Kennedy and Smith are also applying the protein synthesis method to slow wave sleep, and Smith is studying plasticity in the primate visual system during postnatal development by means of the same method. Both studies are being carried out in rhesus monkeys, and progress is very slow because of the limited availability of this species. Nevertheless, the results thus far indicate that there may be interesting changes in protein synthesis that may shed light on the underlying mechanisms. Definitive conclusions must await enlargement of the series to allow valid statistical analyses.

Smith and Ingvar have completed their studies on changes in local cerebral protein synthesis in normal aging in rats. These studies showed selective depressions with age in local rates of protein synthesis, particularly in the primary sensory systems and in the locus coeruleus. Some of the structures affected are known to exhibit decreased functional activity and energy metabolism and also neuronal loss with age. The changes in protein synthesis may be causally related to the other changes. Reports of these studies are currently in press.

Porrino has collaborated with Dr. I. Kopin and S. Burns in the application of the deoxyglucose method to the MPTP-induced model of Parkinsonism in monkeys. She has found marked decreases in glucose utilization selectively in the substantia nigra (pars compacta), subthalamic nucleus, and external segment of the globus pallidus. No effects were found in dopaminergically innervated limbic areas, such as the nucleus accumbens. L-DOPA treatment, which produced therapeutic effects in the Parkinsonian monkeys, produced increased glucose utilization throughout the motor and premotor cortex, in motor nuclei of the thalamus, and especially in the subthalamic nucleus and globus pallidus. This is in contrast to the complete lack of effects of L-DOPA administration to normal animals. These results emphasize a potential role of the globus pallidus-subthalamic nucleus circuit in the organization and performance of normal movement and may provide new insight into the mechanism of action of L-DOPA in the treatment of Parkinsonism.

Unfortunately, a major portion of the Section's biochemical resources and manpower has been forced to be diverted to studies designed to examine and, if possible, to respond to attacks on the validity of the deoxyglucose method. Such attacks have occurred intermittently since the first report of the method, but they have been largely evaluated and discounted by the scientific community. The issue was brought to a boil by the publication in Science of an article by a science journalist, not a scientist, who presented a one-sided account of the complaints of the opponents of the dexoyglucose method. The opponents of the deoxyglucose method are almost entirely members of two groups, both of whom are promoting and attempting to attract attention to their own alternative methods for measuring local cerebral glucose utilization based on the use of [^{14}C]glucose. These methods have such obvious deficiencies that they have been largely ignored by the scientific community. Their proponents attribute the neglect of their methods to the widespread acceptance and, indeed, acclaim of the deoxyglucose method, and they have been carrying on a campaign to find something, anything, that might discredit the deoxyglucose method. They have focused their attention on the alleged role of glucose-6-phosphatase activity, despite the results of three decades of neurochemical research, repeatedly stated in almost every textbook of biochemistry, that there is little, if any, glucose-6-phosphatase activity in brain, heart muscle, and skeletal muscle. They have published, in most cases in marginal journals, studies alleging to show errors in the deoxyglucose method due to glucose-6-Pase activity in brain. It has been necessary to examine these claims by experimental means, and these activities have occupied most of this year's efforts of Nelson, Lucignani, Sokoloff, Kaufman, and Atlas, with part-time assistance of Tannenbaum, Porrino, and Kennedy, and almost everyone else in the Section. They have found in every case that the published reports of deficiencies in the deoxyglucose method due to glucose-6-Pase activity were faulty and erroneous because of errors in the handling or interpretations of the experimental data or gross artifacts that were ignored or unrecognized by the investigators. In fact, in at least one case, the reported results were so obviously manipulated to suit the prior conclusion that it taxes the limit of credulity to accept that it was due merely to negligence or ignorance. These studies, which refute all the criticisms, are being continued in order to round them out to sufficiently polished form for publication in major biochemical and neurochemical journals.

Section on Myelin Chemistry
Marian W. Kies, Ph.D., Chief

This Section has continued to devote its interest to the study of myelin, myelin basic protein, and their respective immune reactions. Dr. Martenson's major interest is protein conformation and structure. He has been able to enlist the collaboration of a very fine group of physical chemists in Australia (G.L. Mendz, W.J. Moore, and L.R. Brown) to do nuclear magnetic resonance studies on fragments prepared by members of our group. Dr. Audrey Stone (currently in Dr. Brownstein's laboratory) has been collaborating also in these studies of conformation by physical methods other than NMR.

The production of highly purified and characterized peptides by proteolytic cleavage has enabled Dr. Martenson, in collaboration with Dr. Alvord's group, to define the specificity of their collection of monoclonal antibodies (Mabs) to BP. In turn, the behavior of these Mabs has provided information on the tertiary structure of BP.

These peptides have also been useful in studying a second immune reaction of BP, induction of EAE in rabbits. It has been known for some time that different species recognize different regions of the BP molecule as being encephalitogenic. Only recently did it become evident that different inbred strains within a species also recognize sites unique to each strain (e.g., Str 2 and Str 13 guinea pigs react to different regions of the BP polypeptide chain).

Dr. Kira has shown that there are two sites in the BP molecule capable of inducing EAE in rabbits. Some rabbits respond to peptides containing residues between #15 and #31. Others react to a site which occurs in the peptic peptide (Res. 45-87). Since rabbits are not inbred, we are unable to distinguish between two possibilities - that all rabbits react to 2 sites or that some individuals react to one site whereas other individuals react to the second site.

Under Dr. Martenson's direction, Mrs. Pedersen has been studying the specificity of the splitting of BP by plasmin, a proteolytic enzyme activated by a soluble product of macrophages. This study is of interest because of the importance of macrophages in the formation of the CNS lesion in EAE.

Dr. Driscoll has been studying mechanisms of autoimmune damage in the CNS. Cells capable of transferring EAE from BP-sensitized donors to naive recipients are manipulated _in vitro_ to ascertain the possible pathways of activation which they must undergo before transfer of EAE is achieved (a hundred-fold activation of the cells with respect to their EAE-inducing capabilities). When BP is added to cultures of BP-sensitized cells IL-2 is produced and utilized and the cells undergo proliferation. This reaction is necessary but not sufficient for enhanced transfer. Other pro-inflammatory pathways must be induced if acute clinical EAE is to develop. Lipopolysaccharide (LPS) or LPS-activated cells apparently provide the necessary pro-inflammatory component and, in collaboration with BP-specific cells exposed to IL-2, transfer severe EAE.

In addition to the cellular immunity and inflammation in acute EAE, chronic EAE has the added component of demyelination. Demyelination can now be induced in animals injected in separate sites with encephalitogen (i.e. BP) and an encephalitogen-free source of central nervous system antigens (i.e. chicken brain). The antigen responsible for demyelination is found in the myelin fraction of chicken brain. A clear understanding of the events responsible for generation of an immune response is necessary before intelligent intervention in the process is possible. This is particularly true of cell-mediated immune responses, including delayed-type hypersensitivity. Delineating the role played by specific immunity, subsequent inflammation and, in the case of the CNS, demyelination, is critical to our understanding of various pathologic states. While _in vitro_ testing is useful for detecting specific immunity, studies on specific immunity leading to inflammation must be done _in vivo_. EAE provides an invaluable system for delineating these responses.

Mrs. Deibler, whose expert handling of HPLC and amino acid analysis is essential to each of the other projects in the laboratory, has been seeking to identify an unknown band which we see repeatedly in electrophoretic patterns of our purified human basic protein (HBP). The presence of this unknown (polypeptide) band was first ascribed to degradation of BP by a trace of proteinase which accompanied the preparation. Having succeeded in removing the proteinase, we still observed the band as a consistent feature of the HBP patterns. It moved slightly ahead of the 1.8 K HBP. Mrs. Deibler has been able to identify the unknown as a mixture of 2 smaller (HBP-related) polypeptides corresponding to Res 18-170, and Res 1-155, which also has an internal deletion near the Trp residue. Res. 18-170 accounts for most of the previously unidentified polypeptide band on the electrophoretic pattern. These smaller "fragments" of HBP may be the result of autolysis _in situ_ and/or defective messenger-RNA processing.

Solving this problem required development of a new procedure for tryptic-peptide mapping of BP. BP is first subjected to limited thrombic digestion which splits the molecule at the Arg-Thr bond (Res. 97-98). The two peptides are separated and each subjected to tryptic digestion. The small tryptic peptides can be separated by HPLC, the elution time of the peaks determined very accurately and each peak collected for amino acid analysis. By this method each peptide can be unequivocally identified. This method will be extremely useful to other investigators interested in BP or other related polypeptides.

Thus the program of the Section is a two-pronged approach to the solution of autoimmunity in the nervous system. What is it about myelin which makes it so vulnerable to the destructive effect of immune reactivity to BP? What is it about BP which makes it uniquely effective at inducing the immune response which is destructive to myelin? We have attempted to answer this circular question by examining the structure and conformation of BP as it exists in aqueous solution and in myelin combined with lipid; by examining the reaction pathways which contribute to CNS damage - cellular immunity, inflammation, and demyelination - and by studying the nature of the fragments of BP which are the products of _in vivo_ degradation processes. These could be related to the BP fragments which are formed by the cellular proteinases liberated by T cells during the BP-activation process.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health
October 1, 1983 to September 30, 1984
Giulio L. Cantoni, M.D., Chief

Fiscal year 1984 has seen the gradual unfolding of a series of administrative action that have brought about an enormous and deleterious change to the structure and organization of the Laboratory. Two independent research groups, headed respectively by Drs. W. Klee and C. Merril have been transferred to other units of NIMH. The space available to the remaining staff in Bldg. 36 has been curtailed drastically with a loss of two thirds our space. These administrative decisions will affect in a negative way the future of the Laboratory and will make it impossible to continue to pursue "a many faceted program of fundamental research" that the recent review by the Board of Scientific Counsellors described as "of remarkable depth and vigor", and with "achievements that are a credit to the National Institute of Mental Health".

Dr. Cantoni and his collaborators, both at NIMH and elsewhere, continued to explore the biological role of transmethylation reactions in many different biological systems by use of analogs of adenosine that interact specifically with Adenosylhomocysteinase. Administration of these compounds leads to intracellular accumulation of potent inhibitors of the biochemical utilization of Adenosylmethionine and provide a novel and valuable tool towards the identification of methylation (or alkylation) processes that are important in a series of different cellular functions such as viral replication, chemotaxis and cellular differentiation. The scientific background and rationale of our approach has been detailed in annual reports of previous years and need not be repeated here (see also project). Whether with the decreased resources available we will be able to continue to develop the broad attack on problems of cell biology that the Scientific Counsellors described as having "great interest and promise for the future" remains to be seen.

In the past year Dr. Klee and his colleagues have succeeded in purifying both the affinity labelled binding subunit of the opiate receptor and the inhibitory receptor coupling protein N_i to states of near homogeneity. The opiate receptor subunit is present in membranes in very small amounts and required 20,000 fold purification. Most of this enrichment was the result of two affinity purification steps using wheat germ lectin - agarose columns, followed by an antibody affinity columns. The antibody was directed against fentanylisothiocyanate, a covalent opioid used to specifically tag the receptor. Final purification was achieved by preparative gel electrophoresis. The coupling protein, N_i, was purified from beef brain membranes by conventional chromatographic procedures from beef brain membranes, a particularly rich source of the protein. The protein behaves as a homogeneous species in the analytical ultracentrifuge and can be shown to dissociate into its subunits on treatment with GTP and Mg^{2+}. Pertussis toxin catalyzes the incorporation of one ADP-ribosyl moiety per molecule of the purified protein. The availability of purified receptor and N_i preparations allow this group to undertake reconstitution experiments. Efforts are underway to prepare antibodies to the receptor and to obtain amino and sequence information with the goal of isolating the gene coding for this important protein.

Dr. Merril and his colleagues developed a new ultrarapid, ultrasensitive silver stain for the detection of proteins and nucleic acids. Previous silver stains relied on organic reducing agents to initiate the formation of metallic silver images while the new stain utilizes light, or photoreduction. This new stain permits visualization of protein patterns within ten minutes after electrophoresis, in contrast to an hour for the previous stains. Clinical application of silver staining to two-dimensional protein patterns from tissues and body fluids, particularly cerebrospinal fluid, has revealed a number of protein alterations in samples from patients with diseases involving the central nervous system. These cerebrospinal fluid protein alternations include: the observation of new immunoglobulin light chain polypeptides in multiple sclerosis, a new polypeptide of Mr 25,000 in Parkinson's disease; the observations of 2 polypeptides Mr 40,000 in 90% of herpes encephalitis patients which were not observed in any of 91 normal cerebrospinal fluids, but were found in 30% of schizophrenic patients; the observation of 2 polypeptides of Mr 27,000 in 1000% of Creutzfeldt Jacob disease patients that were absent from all normal spinal fluid. Prospective studies are underway to test the diagnostic potential of these polypeptides. These polypeptides are currently being isolated for sequence analysis, with the goal of synthesizing DNA probes for the genes coding for these proteins.

Work in the Section on Alkaloid Biosynthesis has been aimed this year chiefly at reducing the backlog of unpublished observations which had accumulated from a variety of investigations during the past several years. Results had been obtained at rates such that preparation of manuscripts had lagged far behind. While this process is as yet incomplete, for each of the research projects much progress has been made in finishing final experimental work and on preparation and processing of manuscripts. Comprehensive statements as to the experimental results obtained and their significance are to be found in the individual project reports. In brief summary, the achievements of the past year fall in three chief areas: a) We have continued our efforts to provide deeper understanding of the biosynthesis of methionine and other essential amino acids of the aspartate family in higher plants by: (i) analyzing regulation of sulfate uptake and resolving a controversy raised by findings in other laboratories as to the anatomical locus of this regulation; (ii) furnishing a comprehensive analysis of the various channels used for uptake of organic compounds and resolving a long-standing controversy as to how amino acids are transported; (iii) bringing into focus a major puzzle as to how threonine biosynthesis is regulated, and providing the means to answer this question by further studies. b) A good deal of time and energy this year was devoted to preparation of a document entitled "Response to Comments of the Board of Scientific Counselors, National Institute of Mental Health, upon the Section on Alkaloid Biosynthesis, Laboratory of General and Comparative Biochemistry, May 26-28, 1983". Preparation of this document was necessitated by what we think were unfortunate and ill-founded conclusions which have led to a 25% decrease in our scientific manpower and otherwise adversely impacted upon our work in a variety of ways. We believe that the response submitted satisfactorily put to rest these unfortunate conclusions. c) The questionnaire study of cystathionine β-synthase deficiency has been virtually completed. The results should open the way to a good deal of progress in the study of this disease in future years. We believe also that the findings obtained will be useful not only to patients with cystathionine β-synthase deficiency, but that they may in addition contribute to understanding the pathogenetic mechanisms and to development of effective preventive therapy for processes which may be important factors now placing very large numbers of persons at increased risk of life-threatening thromboembolic disease.

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY
National Institute of Mental Health
October 1, 1983 to September 30, 1984
Ichiji Tasaki, M.D., Ph.D., Chief

The present annual report will be the last from this laboratory. The Laboratory of Neurobiology will cease to exist as an independent research unit in the immediate future.

The goal of the research activities in the Laboratory of Neurobiology has been to elucidate the nature of the excitation processes in nerve fibers and cells on a macromolecular basis. Both physicochemical and biochemical approaches have been employed to achieve this goal. Since these excitation processes form the basis for the physiological function of the nervous system in normal as well as abnormal conditions, studies of these processes are relevant to the program of the Institute. During the last year, the following three research projects are being carried out.

1. Detection and interpretation of mechanical changes in the nervous system associated with the process of excitation. (I. Tasaki, Y. Nakaye. and P. Byrne)

Since the discovery of the phenomenon of swelling of invertebrate axons during excitation in this laboratory, we have established the following basic properties of the mechanical changes in nerve fibers and cells. (a) The peak of swelling of a fiber (i.e., lateral expansion) coincides fairly accurately with the peak of the action potential recorded at the site of mechanical recording. (b) The swollen state of the axon travels longitudinally at the speed of the nerve impulse. (c) Conduction of a nerve impulse along an axon is associated with a decrease in the length of the axon. [This decrease is an expression of "swelling-retraction" well-known in colloid and polymer chemistry.] (d) A variety of neurons and nerve cells in the vertebrate nervous system swell in a similar manner when they are excited.

During the year just past, we have expanded our field of investigation to include several sensory systems and synapses. The major new findings obtained are as follows:

(1) Isolated retinas of the squid, crab, lobster and bullfrog were found to exhibit rapid mechanical changes when stimulated by light. These changes take place on the same time-scale as the development of electroretinogram (ERG). By combining measurements of mechanical changes with ERG and impedance change analyses, the mechanism of sensory transduction in invertebrate eyes has been investigated. A study of the bullfrog eye by using the same techniques is now in progress.

(2) Sympathetic ganglia of the bullfrog were found to swell in response to preganglionic, as well as postganglionic, stimulation. An antidromic volley of impulses produces rapid swelling that reflects the process of action potential production of the ganglion cells. The mechanical changes

produced by an orthodromic volley was shown to have two components: (a) swelling of the preganglionic fiber terminals, and (b) swelling of the somas of the postsynaptic neurons. In deeply curatized preparations, swelling of the neurons somas associated with the fast excitatory postsynaptic potential could be recorded.

There is little doubt that the phenomenon of swelling described above is directly related to the rise in the membrane conductance. Both in the invertebrate photoreceptor cells and frog sympathetic ganglion cells, the membrane conductance is known to rise when excited. Further studies of physiological properties of the nervous system by using mechanoelectric transducers are expected to lead us to a better understanding of the sensory and synaptic mechanisms.

2. Biochemical studies on the mechanism of nerve excitation. (J. Baumgold and I. Zimmerman)

In nerve and muscle cells, action potentials are mediated by specialized membrane proteins usually referred to as ion channels. One of these proteins, the sodium channel protein, has now been well characterized biochemically in this and in several other laboratories. The current work represents the continuation of a two-year old project and details the last findings on this project.

During the course of ontogenic development, nerve and muscle cells gradually acquire sodium channel protein. In order to understand how these very large proteins are assembled in the plasma membrane, we followed the development of two distinct binding sites on this protein, using two separate and specific neurotoxins: ^{3}H-saxitoxin and ^{125}I-scorpion toxin. We found that in early stages of development, rat brain synaptosomes had twice as many ^{125}I-scorpion toxin sites as ^{3}H-saxitoxin sites, whereas at later stages of development, the synaptosomes had twice as many ^{3}H-saxitoxin sites as ^{125}I-scorpion toxin sites. Despite this changing stochiometry, no biochemical differences were detectable in these proteins.

Following a change in laboratory affiliation, the following project was undertaken:

Since abnormal levels of muscarinic receptor proteins have been found in depressed patients and since clinical depression is genetically linked, we have initiated a program designed to identify abnormalities in the genes' coding for muscarinic receptor proteins in depressed patients. In collaboration with Dr. E. Gershon (Laboratory of Neurogenetics, NIMH) we have made substantial progress in purifying the muscarinic receptor protein. Once purified, we plan to microsequence part of this protein from which we will be able to construct an oligonucleotide probe which can be used to identify and isolate genes for the muscarinic receptor protein.

3. Regulation of protein and enzyme function by modulation sites on complex carbohydrates. (A. L. Stone and M. M. Dohadwala)

The process of cell migration and differentiation in the developing mammalian CNS involves numerous factors whose roles are yet to be determined. One such factor, found recently in media conditioned over various cell cultures, is heparin sulfate proteoglycan. We have been investigating the role of heparin sulfate proteoglycan, GAG, and conditioned media in neuronal development. Our preliminary goal was to find a suitable neuronal culture model. Adrenal chromaffin cells (AMC) of embryonic and adult rats were reported to respond to various conditioned media by increasing neurite extension and neuronal development. Our experiments utilizing various conditioned media with and without nerve growth factor and GAG factors failed to elicit a change in the cell characteristics of the readily attainable adult bovine AMC, however. We have also investigated a "transformed" neuronal cell culture supplied by Dr. Hayden Coon (NCI). This had been isolated from the cerebellar area of the 13-day old rat embryo and transformed by SV40. These neuroblasts were always arrested in a partial development toward mature neurons. Thus far, we have found a low incidence of a more fully developed neuronal cell in the cultures treated with the experimental substrata and will continue to explore the potential usefulness of this model. We have obtained preliminary results using primary cultures of dissociated cells of the mammalian embryonic cerebellum. These cells appear to be stable in control cultures up to 10-12 days and may serve well as a model.

We have continued to characterize the molecular properties of oligosaccharide segments of heparin and heparin sulfates. Our proposed sequence is supported by the experimental and computer-simulated metachromatic reactions of the decasaccharide.

ANNUAL REPORT OF THE LABORATORY OF NEUROCHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
OCTOBER 1, 1983 THROUGH SEPTEMBER 30, 1984

Seymour Kaufman, Chief

During the last year, one of the major efforts of the Laboratory of Neurochemistry has continued to be a study of the mechanism of regulation of the activity of hepatic phenylalanine hydroxylase. One of the reasons for this emphasis is that it is known that a normal functioning phenylalanine hydroxylating system is essential for normal brain development. Another reason is that this enzyme serves as a useful model for understanding the regulation of the other two aromatic amino acid hydroxylases, tyrosine and tryptophan hydroxylases.

We have advanced our knowledge of the regulatory behavior of phenylalanine hydroxylase by demonstrating directly for the first time that the enzyme exists in a low activity state and a high activity state, each with characteristic physical properties, in addition to differences in their catalytic properties. When the enzyme is activated by incubation with its substrate, phenylalanine, or with a phospholipid such as lysolecithin, there is an increase in the enzyme's fluorescence which closely parallels the increase in hydroxylase activity. By contrast, the pterin coenzyme, tetrahydrobiopterin (BH_4), which can deactivate the enzyme, was found to quench the fluorescence. These changes in the enzyme's fluorescence are the physical correlates of the enzyme's change in catalytic properties. Our results provide strong support for a two state model of the enzyme's behavior, with activators stabilizing one state or conformation and deactivators stabilizing the other state of the enzyme.

We have during the last year proved that the pterin intermediate that is formed during the course of the hydroxylase-catalyzed oxidation of phenylalanine to tyrosine, which we discovered earlier, has the structure that we had originally assigned to it, namely, that it is the 4 α-carbinolamine derivative of the corresponding tetrahydropterin. These results provide additional support for the idea that a 4 α-hydroperoxide derivative of the tetrahydropterin is also an intermediate in the hydroxylation reaction. We have also shown that phenylalanine hydroxylase stimulating protein, which we had purified to homogeneity from rat liver, several years ago, is an enzyme that catalyzes the conversion of the 4 α-carbinolamine intermediate to the product, the corresponding quinonoid dihydropterin.

During the last year we have made important progress in understanding the mechanism of de novo biosynthesis of the hydroxylation cofactor, BH_4. It was previously believed that BH_4 is synthesized from guanosine triphosphate via a series of pterin intermediates that are all at the dihydro level of reduction. There was some evidence, for example, which indicated that sepiapterin and dihydrobiopterin, both dihydropterins, are intermediates in the pathway and that sepiapterin reductase, the enzyme that catalyzes the interconversion of these last two dihydropterins, is involved in BH_4 synthesis. We have now shown that dihydropterins are probably not intermediates in the biosynthesis of BH_4. Rather, we have evidence that the intermediates are all at the reduction level of tetrahydropterins. Consistent with this conclusion, neither sepiapterin nor dihydrobiopterin appear to be intermediates. Even though these two known substrates for sepiapterin reductase are not involved, we have strong evidence that sepiapterin reductase is an essential enzyme in BH_4 synthesis.

These results have radically changed accepted ideas about how BH_4 is synthesized and have brought us considerably closer to being able to precisely localize the step that is blocked in the most prevalent form of hyperphenylalaninemia caused by defects in BH_4 synthesis.

We have found that the anti-inflammatory drug, indomethacin, inhibits the stretch-induced stimulation of protein synthesis in chick-embryo muscle. These results, which are consistent with effects of this drug reported for another *in vitro* model system for studying muscle hypertrophy, suggest that prostaglandin may play a role in the stretch response and, therefore, in the development of hypertrophy. They also raise the possibility that arachidonic acid may be released from membrane phospholipids in response to stretch and that this prostaglandin precursor could also be part of the chemical stimulus that leads to hypertrophy. This idea would be congruent with our earlier finding that phosphatidyl inositol turnover increases early during the development of skeletal muscle hypertrophy.

We have completed this year a detailed comparison on a patient with hyperphenylalaninemia due to defective BH_4 synthesis of two types of therapy, one involving the administration of a BH_4 analogue, 6-methyltetrahydropterin ($6MPH_4$) and the other involving the administration of the neurotransmitter precursors, DOPA and 5-hydroxytryptophan. We found that whereas the latter treatment was partially effective in improving this patient's neurological development and her severe central deficiency of monoamines and their metabolites, $6MPH_4$ was ineffective in improving the neurological or the neurochemical abnormalities. The results of this study, when taken together with those of our earlier work, lead to the following important conclusion: patients with defective BH_4 synthesis are a heterogeneous group; in those patients who are responsive to pterin therapy, this therapy will likely be more effective than neurotransmitter replacement therapy; in those patients who are completely unresponsive to pterin therapy, treatment with the neurotransmitter precursors can still be partially effective. A combination of both types of therapy may be the initial treatment of choice.

Dr. Neville and his colleagues have continued their studies of the use of receptor-mediated transport to develop a new class of highly specific drugs. They have now used a mixture of anti-T cell monoclonal antibody-ricin conjugates, which they have prepared, to treat human donor marrow during bone marrow transplantation in order to eliminate graft-versus-host disease. To date, no moderate to severe cases of graft-versus-host disease have appeared indicating perhaps a 50% reduction of this morbid condition. Preliminary results have been highly encouraging. This approach should facilitate the use of bone marrow transplants in the treatment of a wide variety of diseases, such as autoimmune diseases, and other disease of the immune system such as acquired immuno deficiency syndrome.

Dr. Nash and his colleagues have continued their studies on the mechanism of genetic recombination. They have studied the topology of the DNA products of *in vitro* site-specific recombination. From this analysis, they have confirmed their earlier hypothesis concerning the wrapping of DNA by recombination proteins and have deduced important features of wrapping. In addition, they introduced a new class of semisynthetic DNA substrates for *in vitro* recombination and have showed that these substrates have promise for dissecting the early steps of the reaction. Finally, they have looked into the mechanism by which a recombination protein is used to regulate gene expression. They find that bacterial protein affects the transcription of DNA into message.

Annual Report of the Laboratory of Neurophysiology
National Institute of Mental Health
Edward V. Evarts, M D. Chief
October 1, 1983 -- September 30, 1984

The broad goal of research in the Laboratory of Neurophysiology (LNP) is the elucidation of the linkages between brain areas involved in controlling mood, movement, attention, preparatory set and memory. The way in which these linkages operate can be discovered only by a combined, interdisciplinary approach that uses the techniques of neurochemistry, neuroanatomy, experimental psychology and neurophysiology. A major focus in this LNP research program is a set of subcortical brain structures referred to collectively as the basal ganglia and a second major focus is the primary and non-primary motor cortex with which the basal ganglia are linked. Inputs to the basal ganglia arise from many different parts of the cerebral cortex and in addition, from the limbic system. Thus, the basal ganglia provide a junction point for limbic inputs underlying mood and cortical inputs that provide signals related to attention, preparatory set, memory and movement.

The three major research groups within the LNP are focusing their efforts on brain structures that feed into the basal ganglia, on the basal ganglia themselves and on the targets of basal ganglia output. Herkenham and his group are investigating the Functional Neuroanatomy of the basal ganglia, with an emphasis on the distribution of receptors that mediate the function of the endogenous opioid peptides. In addition, Herkenham and his colleagues are investigating plasticity of the anatomical structure of the basal ganglia in relation to the effects of early drug exposure (occuring in utero). The second major group within the LNP, headed by Wise, is studying Information Processing in Sensorimotor Cortex with special attention to the activity of the cerebral cortex in behavioral situations in which the critical dimension is the preparation and readiness for movement rather than the actual execution of movement. Wise has worked out a number of techniques that reveal the high degree of involvement of non-primary motor cortical areas in preparation for movement. Future work will be aimed at comparing different non-primary motor areas with an aim of determining how the ones receiving inputs from basal ganglia differ from those with cerebellar inputs. The third research group in the LNP, headed by Evarts, is involved in research on Motor Psychophysics, and carries out studies in human subjects as well as in subhuman primates. The work in monkeys deals with primary motor areas that are directly involved in the execution of movement as well as in research on cell discharge in basal ganglia. The work of this group has also been directed to studies of motor function in neuropsychiatric patients. The aim of the latter studies is to develop techniques that may bridge the gap between basic laboratory work in animals and an understanding of the nature of psychomotor disorders in neuropsychiatric patients.

In what follows there will be a summary of the work in each of these three major divisions of the LNP research program.

I. Functional Neuroanatomy:

Work in this area is guided by Miles Herkenham and carried out in collaboration with Sandra Moon Edley, Ronald Hammer and Charles Gerfen. The projects now in progress may be divided into two groups, one involving neurochemical approaches to neuroanatomy and the other dealing with metabolic correlates of brain activity. In the summary that follows these two approaches to "dynamic brain structure" will be dealt with in two parts.

Neurochemical Investigations:

Over the last decade a major thrust of neuroscience research has been the identification of neurotransmitter, neuromodulator and hormone receptors in the brain. An understanding of receptor function requires knowledge of the biochemistry and pharmacology as well as the neuroanatomical localization of receptors. Identification of receptors by pharmacological criteria has been carried out in collaborative studies with Dr. C. B. Pert and members of the Clinical Neuroscience Branch of the NIMH. We next seek to identify the neuronal circuitry that is associated with these receptors by comparison with known anatomical pathways and by immunohistochemical identification of transmitter-specific connections. Other main objectives are to understand the role of a receptor or receptor subtype in any given region by determining receptor density, plasticity in tests of developmental time course or pharmacological manipulation, and altered distribution in neuropathological tissues.

To permit these analyses, Herkenham and his associates have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue sections. Using emulsion autoradiography, the receptor distributions can be compared with underlying morphology seen in the stained sections. Stains for other features of brain chemistry and functional heterogeneity can be applied to the same or adjacent sections. Using film autoradiography, the receptor patterns can be analyzed by a densitometer for computer-assisted quantification of receptor densities.

The group has been particularly successful at detecting subtle differences in the distributions of receptor subtypes and precise correlations with other anatomical, chemical and functional markers, including those for cells and fibers, acetylcholinesterase, labeled pathways, catecholamine fluorescence and 2-deoxyglucose metabolism. The technique is well-suited to study fragile tissues and tissues that require sub-optimal binding conditions, and so the group has succeeded in studying fetal development and the binding of several "novel" peptides.

Concurrent visualization of dopamine, autoradiographically-labeled projections and receptor distributions in the adult rat have led to understanding of the neurochemical and connectional compartmentalization within the mammalian striatum. The ontogenetic co-development of striatal opiate receptors and dopamine heterogeneity has been examined also. To investigate whether dopamine receptor function is important for the normal formation and maintenance of opiate and dopamine receptor distributions, dopamine receptors have been chronically blocked by the antipsychotic, haloperidol, during the prenatal stage when striatal opiate and dopamine heterogeneity develops. Studies on mature striatal receptor systems are in progress to investigate several novel dopamine antagonists that produce varying degrees of antipsychotic effects and movement dysfunction. Ultimately, we might hope to determine the role that opiates and related neurochemicals play in human brain function, and separate out the striatal chemoarchitecture that is involved in some psychiatric and neurologic syndromes.

Metabolic Correlates of Functional Activity.

We have developed techniques which permit us to use $[^3H]$2-deoxy-D-glucose (2-DG) rather than $[^{14}C]$2-DG as a metabolic marker of glucose utilization. The use of $[^3H]$2-DG improves resolution, since the particles of ^3H are less energetic than those of ^{14}C and form an image closer to their source.

Our studies of phencyclidine-induced changes in cortical metabolism elucidate the mechanism of action of phencyclidine analogs in the central nervous system. Metabolism in regions of limbic cortex is stimulated by these drugs while sensory cortical zones show decreased metabolic activity. The ketamine-induced shift in cortical metabolism may reflect a functional disconnection from peripheral sensory input and/or enhanced internal (corticocortical) and limbic processing. These altered states may occur in humans when phencyclidine (PCP) is taken as a drug of abuse.

We have also begun to investigate the functional role of monoamine neurotransmitters in the extrapyramidal motor system, using 2-DG techniques. Depletion of monoamine input to the striatum by chronic reserpine administration causes a metabolic activation of the globus pallidus and the lateral habenula, structures in the extra-pyramidal circuitry. Systemic injection of L-DOPA, a dopamine precursor, results in a rapid reversal of these metabolic alterations. Current work explores the cellular mechanisms underlying these metabolic changes in the extrapyramidal motor system.

II. Information Processing in Sensorimotor Cortex:

The second major area of research within the Laboratory of Neurophysiology is being carried out by Steven Wise and his colleagues within the LNP, John Donoghue and Von Jennings.

One of the major goals of the work being carried out by Wise, Donoghue and Jennings has been to obtain an understanding of the successive transformations of information leading from sensory input to motor output. Such transformations must occur, for example, when a visual stimulus located at a particular point in space is the target for a visually guided limb movement. The visual information must be used to select patterns of muscle discharge that will guide the limb towards the visual target. It seems likely that the transformations involved between visual input and motor output involve passage of nerve impulses between visual cortex and the premotor and motor cortex as well as between these cortical areas and basal ganglia. Wise has developed experimental paradigms that enable him to examine the changes in discharge patterns of neurons in the premotor and precentral motor cortex during a variety of visually guided movements. Similarly, somatic sensory inputs are important in the guidance of voluntary movement, and Jennings is conducting experiments aimed at examining the role somesthetic input to precentral motor and somatic sensory cortex. And since all such transformations between sensory input and motor cortical output depend upon the intrinsic information processing of small modules of cerebral cortex, Donoghue has been examining the relationship between input and output in the somatic sensorimotor cortex of rats.

Thus the work of Wise and his group on cortical information consists of three main projects:

1) Hierarchical Organization in Motor Control Systems
2) Somatic Sensory and Motor Activity of Cortical Cells
3) Input-Output Organization in Somatic Sensorimotor Cortex

The work on Hierarchical Organization has a combined neuropsychological and neurophysiological orientation that is designed to provide information as to the way in which frontal lobe nerve cells control complex, highly skilled voluntary movements and the preparation for such movements. The studies of Somatic Sensory and Motor Activity use neurophysiological techniques to examine information processing in somatic sensory and motor areas. These studies involve a detailed study of single cell activity in the cerebral cortex as well as muscle activity in response to perturbations of the limb. The work on Input-Output Organization seeks to apply sophisticated electroanatomical techniques (intracellular injections of tracers into antidromically identified cells) to studies that concurrently provide information as to the behavioral and pharmacological properties of the cells. In the following summary, each of these three approaches will be considered.

Hierarchical Organization in Motor Control Systems: One general objective of this project is an improved understanding of the organization of the entire motor cortex and its role in the cerebral control of movement. The entire motor cortex is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery. This project is devoted to gaining a better understanding of the cortical fields involved in the sensory guidance of movements and the linkage between sensory signals and motor behavior.

The initial focus of Wise's study of the hierarchical organization of motor systems is the premotor cortex and, in this work, monkeys were conditioned to depress the central key of three keys. After a period of time, either the left or right key became illuminated. Three experimental conditions ensued: (a) the key remained illuminated and served as the target for the subsequently triggered movement, (b) the light was turned off before the monkey was allowed to execute the movement, forcing the monkey to remember the proper target, or (c) the target light was switched before the monkey was allowed to execute the movement. This experiment was designed to test the relationship of neurons in PM to the motor set of the animal, even when the signals are absent or the motor set changes during the course of a behavioral trial.

The results of this study have provided the first clear demonstration of "set-related" (in contrast to movement-related) activity in premotor cortex. It was found that whereas many neurons in premotor cortex markedly change activity before the onset of a voluntary movement, a substantial population of neurons are clearly related to planned motor activity. Several experiments have been and are being designed to further test this proposition and to elucidate the properties of these neurons under a variety of experimental conditions.

Somatic Sensory and Motor Activity of Cortical Cells: This second phase of the work involves a study of the role of sensory inputs to the cerebral cortex in the control of motor behavior in primates. The first part of the project consists of a comparison of neural activity in the somatic sensory cortex (SI) and the precentral motor cortex (MI). The second part of the project is a detailed examination of the signals which the peripheral receptors are sending to the cortex during perturbation of voluntary movements. This work involves a detailed examination of muscle activity (EMG) in relation to such perturbations, as well as single-unit activity in the cerebral cortex. These experiments should provide important clues concerning the role of peripheral inputs to MI in the initiation and control of movement. This work provides an important interface with the motor psychophysics group in the LNP, and part of this project involves a collaborative study of Jennings and Sanes on the role of sensory input in generating characteristic patterns of muscle activity before and during relatively rapid movements.

Comparisons of MI and SI in this project reveal a difference between the two areas: in SI neurons are found with a non-muscle-like relation to force and position. A muscle which is more active when the forearm is supinated is always more active when supinating force is applied to an immovable object (an isometric force). MI cells are invariably like muscles in this regard. In SI, however, a substantial proportion of cells that are active with supinating isometric force became more active with the forearm pronated. The presence of both muscle- and non-muscle-like neurons in SI is consistent with that region's postulated role in sensory processing. In order to interpret inputs which signal a mixture of position and force information it is necessary to have two inputs which code position and force in different ways. In contrast, the lack of non-muscle-like neurons in MI is consistent with its role in motor control. MI output does not uniquely specify force or position independently. Instead it appears to specify muscle tension, which depends on both force and position.

Much work in the past year has been devoted to an examination of the neural and muscular events that follow the interruption of a voluntary movement. This problem is important to the understanding of the mechanisms by which the motor and somatic sensory cortex may compensate for obstruction of intended movements and the role of sensory feedback in cortical motor control.

Input-Output Organization in Somatic Sensorimotor Cortex: The third area of the work of this group is designed to examine the input-output organization of neurons in the motor cortex. Rats have been chosen as the primary experimental animal since they are widely used in the pharmacological, immunocytochemical and electron microscopic studies with which we wish to correlate our neurobiological and behavioral data. The project has four parts: (1) Neuroanatomical and neurophysiological techniques have been employed to characterize the primary motor cortex (MI) in rats and determine its inputs from and its outputs to other neural structures. (2) Single-cell recording techniques have been used to record neuronal activity in the forelimb area of MI cortex in awake, behaving rats. In these experiments, the relationship of the neuronal activity to motor output has been examined during the stimulation of ascending noradrenergic inputs to the motor cortex. (3) Intracellular injection of a tracer, horseradish peroxidase has been combined with retrograde transport methods in order to visualize the intracortical distribution of neuronal processes belonging to identified intracortical cell types and their possible connections with projection neurons. And (4) the distribution of putatively glutaminergic neurons has been demonstrated with immunocytochemical methods. In both primates and rodents, most of the putatively glutaminergic neurons are located in cortical layers V and VI, suggesting that they are corticofugal projection neurons.

Each of these four approaches has led to new information about the input-output organization of motor cortex in rats. The MI cortex can be defined cytoarchitectonically in rats, as in other species, and it has been found that the rat's MI cortex is characterized by the same major features as in other mammals. For example, it had previously been thought that the output from the rat motor cortex to the spinal cord terminates only in the dorsal horn of the spinal gray matter. However, Donoghue and Wise discovered that the rat's MI corticospinal projection is instead directed to the ventral and intermediate horns of the spinal gray matter, just as it is in primates. This connectional feature along with its cytoarchitecture, its overall pattern of afferent and efferent connectivity, and its low threshold for electrical excitability indicates that the rat MI cortex represents a valid model for the study of motor cortex in general. Single-unit recordings from unanesthetized, behaving rats confirm this view. Cells in MI are very similar in their discharge properties to those reported for primates and, as in primates, the MI cortex is divided into two subregions: The rostral part of MI receives sensory inputs primarily from muscle and joint receptors whereas the caudal part receives peripheral inputs mainly from cutaneous mechanoreceptors.

It is now possible to take advantage of the similarities between motor cortex organization in rodents and primates to perform experiments impractical or inadvisable in primates. Preliminary data are available for two of these experiments: (1) To identify the effects of "modulatory" inputs to the cortex, Donoghue electrically stimulated the locus coeruleus, a brainstem nucleus that contains the cell bodies of origin of the noradenergic inputs to cortex. During the performance of a motor task, movement-related activity of many of the MI units is enhanced after stimulation of the locus coeruleus. And (2), examination of the axonal arborizations of multipolar interneurons reveals that these cells have extensive intracortical connections with pyramidal tract neurons. This finding suggests that these interneurons have an important role in information processing within MI, and it is possible that they control the output signals from projection neurons to subcortical targets. The interdisciplinary approach typified by this project holds great promise for advances in understanding of the structural, neurochemical, and physiological mechanisms of behavior, especially since rats are a species in which all of these disciplines can be practiced readily.

III. Motor Psychophysics:

This third major LNP research area is directed by Evarts, and has two facets. The first involves study of activity of nerve cells in monkey cortical and subcortical structures. The activity of these nerve cells is observed in association with voluntary movement, and the data obtained provide information as to the mechanisms underlying control of movement by the brain. The second involves studies of motor function in patients with neuropsychiatric disorders as well as in healthy control subjects and is aimed at understanding the basis for motor abnormalities in neuropsychiatric illness.

Brain Activity During Movement: This work involves utilization of single neuron recording and operant conditioning techniques in behaving monkeys to study brain mechanisms underlying voluntary movement. Cerebral control of voluntary movement depends upon the integration of internally generated central programs with peripheral inputs arising from sensory receptors. Understanding, treatment and prevention of movement disorders in neuropsychiatric patients requires knowledge of the sites at which this integration takes place. Two cerebral structures

(Sensorimotor Cortex and Basal Ganglia) are of particular significance in control of voluntary movement, and current LNP work is aimed at discovering how these two structures interact. Work on the basal ganglia is of particular significance in connection with clinical disorders, since it provides techniques for discovering how toxic substances produce Parkinson's disease and how therapeutic agents exercise beneficial effects in Parkinson's disease. Work on volitionally-related activity in sensorimotor cortex provides information as to the way in which the central set and expectancy of the subject may modify processing of afferent input information and generation of output signals to spinal cord.

State or set-related responses in putamen neurons

Previous microelectrode recordings in monkeys have shown that putamen neurons related to voluntary movement are silent in the absence of movement and, conversely, that tonically active putamen neurons appear unrelated to movement. Recent work has confirmed that tonically active putamen neurons are unrelated to body movement per se, but has shown that under certain circumstances such neurons may exhibit highly reliable responses to external events. An auditory stimulus (the click of a solenoid valve) elicited short-latency responses in this type of putamen neuron when the stimulus was a cue for juice delivery and its consumption, but such a stimulus failed to elicit responses when juice delivery had repeatedly failed to follow the sound, with the result that the sound no longer triggered movements associated with consuming the juice. Responses of tonically active putamen neurons were observed in three behavioural conditions: (1) self-paced movement, in which a series of elbow movements resulted in a solenoid click and a juice reward; (2) free-reward, in which click and juice occured at regular intervals (every 6 s) while the arm position was fixed; and (3) no-reward, which was similar to the free-reward condition except that the tube conveying the juice was occluded so that the solenoid click was no longer followed by juice. In the no-reward condition licking was extinguished after the first few unrewarded solenoid clicks.

A sequence of 40 consecutive rewards during self-paced movement was followed by 40 consecutive free-rewards. There was no apparent difference between solenoid-evoked activity in self-paced movement and in free reward, showing that presence or absence of arm movements before the solenoid click made virtually no difference in the neuron response. Then, without any alteration to the 6-s intervals between solenoid clicks, the sequence of 40 no-reward clicks was started. The monkeys reacted to the first few unrewarded clicks after the long sequence of 80 rewarded clicks with the motor responses (head torque and sublingual EMG) that would have been appropriate for consuming the juice, but these motor responses were quickly extinguished as the series of 40 consecutive unrewarded solenoid clicks proceeded. (It should be noted that the monkeys had had much experience with these three sequences, and had learnt that an unrewarded click signalled many more to come.) Tonically active putamen neurons that had been responsive to the click lost their responses within a few repetitions of the unrewarded solenoid click, showing that the characteristic responses of these cells to the solenoid click depended on the state or set of the animal towards consumption of the reward. Though dependent on this state or set of the monkey, the responses in the tonically active neurons were not related to licking movement per se, i.e. the cell did not change its discharge rate in relation to movements not preceded by the auditory stimulus. These neurons responded to the solenoid click with single impulses well in advance of the first in the sequences of licking movements and showed no apparent relation to the subsequent successive licks. By contrast, typical movement-related neurons had bursts of discharge with each of the series of self-paced arm movements or licking movements.

Tonically discharging putamen cells with set-dependent responses were observed throughout the entire putamen, and unlike the movement-related neurons they appeared to be independent of any somatotopic organization. It may therefore be inferred that the single impulses evoked in tonically active cells about 60 ms after the solenoid click resulted in a synchronous event within a large part of the putamen.

Looking at the single impulses evoked in tonically active putamen cells, and contrasting these single impulses with the intense movement-related activity of the phasic putamen cells, one might be inclined to minimize the functional significance of the tonically active cells. However, the dopaminergic nigrostriatal neurons are also tonically active and lack phasic relations to movement, but their importance is well established. By analogy, the tonically active putamen cells might also have great functional significance in spite of the small number of stimulus-evoked impulses. It is clear that a high priority in further work on the putamen will be the neurochemical and morphological identification of the tonically active putamen neurons and a further analysis of the state-dependent or set-dependent responses.

Human Studies:

Sanes and his colleagues have recorded muscle activity and kinematics of limb position while (1) subjects manually match a target display with either a rapid or slow movement with a handle whose displacement controls a visual display or (2) maintain postures when limb position is passively changed. Movement amplitude, presence or absence of visual feedback of position, disturbances of the subject's movements and changes in sensory input are independent variables. A second approach records a variety of psychomotor variables from patients with Parkinson's disease, cerebellar disorders, and peripheral sensory neuropathy.

The importance of afferent information in motor control is readily apparent in patients with neurological disorders such as peripheral neuropathy resulting in sensory loss, but tests of motor skills have not yet developed to the point of providing us with useful quantitative indices of the way in which afferent input is used in controlling motor output. Use of physiological techniques combined with motor psychophysics and modern computer displays, together with collaborative work with clinical neurologists, is allowing the present project to clarify a number of longstanding problems of sensory control of movement. Thus, it has been found that the trajectories and postural responses of patients with cerebellar ataxia and sensory neuropathy are different from normal subjects in that there is a greater variability in the dynamic and static responses for a wide range of movement sizes. The deficits in movement trajectory have similarities and differences in the two patient group. Patients with either a sensory neuropathy or cerebellar disease exhibit ataxia, but the movement disorder is differentially responsive to visual guidance. That is, cerebellar patients show reduction of learned movement inaccuracies when movements are performed without vision, whereas patients with a large-fiber sensory neuropathy perform worse when vision is occluded. With respect to maintenance of hand position both patient group show instabilities, but the tremor exhibited by patients with cerebellar disease probably reflects the dysfunction of somesthetic feedback loops whereas for patients with sensory neuropathy the deficits appear when visual guidance is removed. This suggests that the "memory" of the motor command degrades quickly in the absence of peripheral feedback. Furthermore, the end point error of movements by patients with sensory neuropathy is extremely sensitive to preloads.

Another procedure to determine motor acuity of normal subjects and patients with neurological diseases involves determining the spatial accuracy of repetitive step movements of various sizes. Both normal subjects and patients with peripheral sensory neuropathy performed large and small movements accurately when visual guidance was available. Movement accuracy deteriorated for both normals and patients when visual guidance was absent, but the patient with sensory neuropathy showed two to four times more error than normal subjects. Furthermore, whereas the error of normal subjects decreased in absolute magnitude when smaller movements were performed, the spatial error exhibited by deafferented patients decreased only a small amount when the smallest movements were performed.

In another group of studies psychophysical methods that measure reaction time performance have been used to evaluate the integrity of motor programming processes in patients with neuropsychiatric disorders. Reaction times are abnormally delayed in parkinsonian patients when task difficulty increases. Although reaction times preceding simple movements were slower in parkinsonian patients the reaction time deficits were particularly apparent when sequential movements were being planned. This may indicate that there is interference between individual "motor programs" in either the retrieval or execution states, in Parkinson's disease.

A second facet of work in human subjects has involved development of a computerized system for obtaining objective and quantitative measures of motor function in patients with neurological disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system has been used to record and subsequently summarize data concerning motor functions in more than 150 patients with parkinsonism participating in clinical trials of the experimental anti-parkinsonian drugs, lisuride, bromocriptine, pergolide and nadolol. Patients with cerebellar disorders and essential tremor have also been studied. To establish normative data for movement variables measured on our system, thirty age-matched normal subjects have also been tested. An additional use of the computer system has been to begin studies of psychomotor performance using the methods devised by experimental psychologists. These studies will be useful to provide objective measures of the organization of motor performance and to assist the clinician in diagnosis and evaluation of patients with sensorimotor disorders.

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects were observed. Parkinsonian patients undergoing treatment with lisuride, bromocriptine, pergolide or nadolol were tested on a regular basis throughout the course of the buildup and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date have been permanently stored in computer files and are accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

These two aspects of the work in human subjects will provide clarification of how tactile and kinesthetic sensory inputs are used to control skilled motor activity, clarification that is essential to the understanding of normal and abnormal motor behavior in humans. In addition, these studies will potentially develop standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patient's progress on medication regimens is particularly suited for objective analysis.

IV. Outlook:

The foregoing sections of this summary have described three major areas of research within the Laboratory of Neurophysiology (LNP). Much of this research is newly undertaken and has involved recently recruited scientists whose skills and training equip them to take maximum advantage of the many promising neurobiological techniques that have developed in the last decade. The LNP has evolved from a laboratory that was primarily oriented toward electrophysiology ten years ago to one that now combines a number of interdisciplinary neuroscience techniques in studies aimed at understanding brain mechanisms in neuropsychiatric disorders: neuropharmacology and neuroanatomy as well as neurophysiology. This evolution is still taking place, and within the past year major progress has occurred in the coordination of research within the laboratory and the formulation of new experiments that focus on mechanisms of higher brain function. To promote this formulation Evarts and Wise prepared a monograph on the subject of a neurophysiology of behavior and higher brain functions under the auspices of the Neurosciences Institute of the Rockefeller University, a newly established organization designed to promote innovation in neurobiological research. This monograph was published in May, 1984, and will provide a powerful impetus to combined behavioral-neurophysiological-anatomical work within LNP as well as within the field of Neuroscience generally.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

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The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); and (4) provision of other technical services in support of the research program.

The Branch is comprised of two sections:

Section on Instrumentation and Computers provides technical support for investigators by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; and (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Section on Laboratory Animal Medicine and Care provides professional advice and assistance to intramural scientists on all aspects of animal health, medical care, and testing and surgical protocols; manages a central primate holding facility; and conducts a program of laboratory animal care.

Additional services provided by the Branch include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Branch personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Branch is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Branch, he first meets with the appropriate Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether RSB will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, RSB will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Branch Chief agree to accept a project, the investigator submits a standard work request form (available from RSB), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The RSB does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Branch's CAN.

SECTION ON INSTRUMENTATION AND COMPUTERS

INSTRUMENTATION

The Branch has a staff of five engineers and six technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 253 projects undertaken this year.

(1) Patient Activity Monitoring System. The Branch has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system.

(a) Monitor. Last year the PAM was redesigned to obtain a four-fold increase in memory capacity and a significant reduction in size. This year the major hardware efforts involved producing this new PAM in large quantities and redesigning the monitor's case to take full advantage of the reduced size of the circuitry. Forty-six new monitors were fabricated, tested and calibrated early this year. A second set of 60 monitors is now in the final phase of fabrication.

In order to maximize the benefits of a smaller case, a contract with a local firm was awarded to design and produce a three piece injection-molded plastic case. The pieces are to snap together, with the main case and end cap forming a water-tight seal. Pre-production sample cases are now being evaluated. While the development cost of this case was high, production quantities of the case will be extremely inexpensive and readily available.

(b) Telecommunications. The PAM data telecommunications system project has resulted in the development of a remote readout terminal for the patient activity monitor. The hardware/software development phase is complete and a printed circuit board is now under development. An important facet of the development program was the incorporation of a new line of low power (CMOS) parts into the hardware design. As a result the power supply requirements were reduced by an order of magnitude resulting in a smaller package and less ventilation demands. The new hardware also eliminated the need for interface circuitry previously required to match the CMOS activity monitor to the HMOS hardware of the previous terminal design. The terminal has the capability to be used in the home of a subject or in an office/laboratory environment. Using a predefined software protocol, the terminal reads the contents of an activity monitor, dials a remote computer facility, sends the data read from the monitor, clears the monitor memory, and hangs up. Initially, the VAX computer managed by the Branch will be the remote (recipient) computer. After receiving the data, the VAX will retransmit it over a phone line to the PAM minicomputer in Bldg. 10. The data can then be reformatted into standard activity files for further analysis.

(c) PAM Checkout Computer. This portable, battery powered instrument was developed last year to provide a means of initializing and testing activity monitors right on the wards. The convenience of this instrument has improved the day-to-day management of the large number of monitors now in use. Nine checkout computers were fabricated this year.

(d) Software. The continuous file plotting program has been updated to enable users to plot outputs on both the Electrohome video display and the HP plotter located in Bldg. 10. Future updates will allow newer, inexpensive dot-matrix printers to also be used. A second software update has expanded the PAM display program to allow users to calculate activity frequency distribution according to user-set parameters.

(2) Neurophysiological Data Preprocessor. A microprocessor system has been developed to replace the custom logic circuitry presently used by the Laboratory of Neurophysiology Data Acquisition System. The new preprocessor records the times of occurrences of 64 different events and 8 different pulses. This information is transmitted to the main processor (a PDP-11 minicomputer) through a parallel interface and the information is coded in such a form as to ensure compatibility with existing software that is used for analysis and display of the data. The preprocessor decreases response time to events and pulses and it frees the main processor for experiment control. A software package has been written for the PDP-11 computer which enables users to add specific modules to present stimuli and/or control the experiment without the need for revising the basic data collection program.

(3) Pulse Generator System. A multi-channel timing instrument (pulse generator system) is a vital part of many neurophysiological experiments. Instruments used within the IRP that were purchased about 15 years ago are no longer manufactured and have become somewhat unreliable. Newer, commercially available units lack the flexibility and convenience of the older devices. RSB has designed a five-channel pulse generator system to fill this void. By employing both analog and CMOS digital design techniques, an instrument with both the required technical specifications and a high degree of operator convenience was realized. The base rate generator section provides a frequency range of .01 Hz to 110 kHz, plus manual and external triggering. Each of the five pulse generators provides a six decade range of pulse delay and pulse width with excellent linearity between ranges. The fifth pulse channel also has pulse train capability. Six of these new instruments were fabricated this year.

(4) EEG Amplifier System. A 32 channel EEG amplifier system was completed and is currently being used for several ongoing research projects including topo0 graphic brain mapping. The design incorporates state-of-the-art integrated circuit components and printed circuit board layouts to produce a reliable, compact, low-cost-per-channel unit. The system consists of a preamplifier and amplifier with an overall gain of 20,000. A flexible design and front panel switches allows

the user control over signal bandwidth, sampling frequency into the computer, and external monitoring by a tape recorder and a 16 channel Grass polygraph.

(5) 30 Channel Electrode Array Amplifier System. A complete system for amplifying and processing signals from a micro-miniature array of 30 gold electrodes is being completed. The electrode array will be used in a variety of experiments to record cultured nerve tissue cell interactions. The signals from the nerve cells are first pre-amplified on a small unit right at the experimental set-up. A separate amplifying unit provides three selectable settings for overall gains of 100, 1,000 or 10,000.

For each electrode, the amplified signal is fed to a comparator with a front panel adjustable threshold level. The output of this comparator triggers a one-shot which is latched and eventually sampled by the computer. Additionally, a multiplexer is provided to display the amplified signal, comparator level and one-shot output on a single channel of an oscilloscope. Design of printed circuit cards for both the pre-amplifier and amplifier/discriminator greatly simplifies the construction and increases the system reliability. A minicomputer system was also specified for this project and will sample each of the 30 latches, record and process the digital data.

(6) Oscilloscope Modification. Modifications to a dual channel B & K oscilloscope were completed which allows the investigator to use an inexpensive oscilloscope as a high speed, dual channel X-Y display. The horizontal and vertical axes, the intensity level, and the chopper frequency are placed under computer control which allows the user complete flexibility in using the oscilloscope for displaying neurological data. All the modifications were placed inside the oscilloscope. A single switch disconnects the circuit modifications and allows the unit to be used in its original oscilloscope mode.

(7) Microphone Amplifiers. Accurate recording of primate vocalizations in large outdoor enclosures required the design of a compact, reliable microphone amplifier to transmit the signal over long cables to the remotely located tape recorder. By utilizing new linear CMOS circuit elements (operational amplifiers, voltage converters, regulators and detectors), an extremely low power, low noise amplifier design was realized. Four of these amplifiers were fabricated; each provides a bandwidth-limited selectable high gain and over 300 hours of operation from a single 9 volt battery.

(8) LED Pulsing System. A light emitting diode system was constructed for experiments where a constant current, variable pulse width light stimulus is needed for retinal response studies. The requirements for this system specify an external input gate that enables a 1 kHz clock. The rising edge of this clock triggers a one-shot that allows a pulse width selection of 10 logarithmic intervals between 100 μsec. and 790 μsec. The selected pulse width is then used to generate a constant current drive for the LED. The constant current source is switch selectable in decades between 10 μA and 100 mA and was designed using a transistor and the LED in a feedback loop of a 741 op-amp.

(9) Microdensitometer. A standard split-viewing Zeiss compound microscope is being converted into a microdensitometer for use in the 2-deoxy-D-glucose autoradiographic method of studying functional brain anatomy. This instrument will produce a density reading from a small central spot (selectable as either .25, .63, or 1.6 mm dia.) within the 18 mm diameter viewing area. A linear

photodiode/amplifier combination will convert the light transmission value within the spot into a proportional voltage for a microprocessor-controlled A/D converter. Corresponding to each transmission value, a logarithmic density value will be obtained from a memory look-up table. Transmission and density values will be simultaneously displayed, each in a four-digit format. Upon foot pedal command, the density value will be printed to facilitate recording of numerous successive readings. The split-viewing ability of the microscope will allow precise areas on the autoradiographic film to be identified by simultaneously viewing the film and a stained slide of the same brain slice section.

(10) Rodent Activity System. A system is currently under development which will monitor the running wheel activity of 72 rodents. The experiments are important in circadian rhythm studies involving light response on free-running hamsters. Six surplus tissue culture boxes will hold 12 cages each. The running wheel activity in each cage will be recorded with a simple microswitch and interface logic controlled by a 16-bit Plessey system 6100 laboratory computer. In addition to the 72 running wheels, a fluorescent light source will present the programmed light stimulus and is located in the upper and lower chambers of each of the six units. The computer will monitor each of these lights by means of photodetectors to verify that the lights were on at the proper time. A total of 84 channels of digital data (72 running wheels + 12 light detectors) will be processed by the computer. The data will be stored on one 10 megabyte Winchester hard disc and also on two 1 megabyte floppy discs. In addition, data on each of the 84 channels will be continuously displayed on a printer/plotter using a strip chart simulator.

(11) Ambulatory Lux Monitor. An ambulatory data acquisition system (Vitalog PMS-8) is being used to monitor the temperature of manic depressive patients. To allow simultaneous recording of the ambient light levels experienced by these patients, a small, micro-power lux meter is being developed as an input transducer for the PMS-8. A wide-angle photodiode with a special photometric filter will be combined with a CMOS logarithmic amplifier circuit to obtain a three decade dynamic range. The microprocessor data processing algorithms employed in the PMS-8 will be modified to handle the light intensity data.

(12) Eye Blink Detector. A device is currently under development to monitor eye blinks in subjects undergoing experimental exposure to light. The light will be administered with a gansfield dome. The device will be used to ensure and to document that the subject's eyes remain open during light exposure sessions lasting approximately one hour. Because light suppresses the pineal secretion of melatonin, it is important to record the total time the eye is open and receiving light. It is planned that a photodetector mounted on a modified eye glass frame detect changing light reflections off the sclera when the eye is opened or closed. When the output of the detector crosses an adjustable threshold, a counter will record the total time the eye is open during the session. A panel light will also indicate to the person administering the test that the eyes are open.

COMPUTERS

Small computers are ideally suited for laboratory research in neurophysiology and psychology. They are used in the laboratory for on-line, real-time interactions, process control, and data acquisition. Recorded data may be stored, combined with other data, reduced statistically, transferred to larger computers for further analysis, transformed for presentation graphically or mathematically, and the results may be printed or plotted. Increasing use is being made of the small computer for processing the text of scientific papers and communications. Data base management is now available for the small computer, as are limited management information systems.

Techniques have been developed for image processing which are applicable to many diverse experimental systems, ranging from autoradiographs of brain tissue sections to the analysis of two-dimensional electrophoresis gels.

Larger minicomputers, the so-called super-mini's, have been reduced in price and are now available for functions formerly performed by larger time-shared systems. These systems allow applications in modeling, curve fitting and statistical treatment that would be prohibitively expensive on large systems.

Inexpensive personal computers are proving useful for dedicated applications. Many scientists are developing software for these computers, which they offer to the scientific community at low cost. PCs will become increasingly useful in the laboratory and their potential should be exploited.

Microcomputers incorporated in the design of biomedical instrumentation provide a savings in design and fabrication time for instruments, and a more flexible system than one based on discrete components.

The Research Services Branch is actively involved in the applications of small computers in the IRP. By integrating the functions of biomedical instrument design and laboratory computer systems with software designed specifically for the research community, the Branch offers computer support services for a broad range of scientific disciplines.

LABORATORY COMPUTERS

The design goal for the laboratory instrument computer is to provide maximum function, tailored to the specific experimental design, with minimum cost. RSB provides consultation on the specification and selection of laboratory computers for new applications; conducts systems studies in collaboration with the scientist; and helps the scientist in the procurement, installation and maintenance of the equipment.

In support of these efforts, RSB maintains two support computers; one in Bldg. 36, and one in the Clinical Center. These systems provide the more expensive equipment necessary for off-line data storage, efficient data processing, communications with DCRT computers, and plotting and printing of the data. The systems are run on an open shop basis and are used for program development, training, and testing the feasibility of new systems for the laboratories.

TRAINING AND SOFTWARE SUPPORT

RSB provides training for the scientist or support personnel who will be programming and maintaining the system. Personnel limitations make it impossible for RSB to provide applications programming, so such programming must be supplied by the laboratory. RSB computer personnel are always available for consultation, training and help in debugging, as well as assistance in the selection of part-time programmers or consultants. Commercial software packages or applications from other research labs are often available, and RSB will evaluate such systems.

RSB maintains a library of procedures which were written specifically for the laboratory computers used in the intramural community. These procedures are designed to be incorporated into the user's programs. In addition, RSB will aid the investigator in writing the difficult time and data dependent sections of real-time programs.

PERSONAL COMPUTERS

The Branch is evaluating personal computers for potential use in both scientific and administrative applications. Potential scientific applications include data acquisition, experimental control, data analysis and display, graphics terminal emulation, and technical word processing. Potential administrative applications include terminal emulation, data storage and retrieval, spreadsheet analysis, and word processing. The Branch has acquired several Apple Macintosh computers and is evaluating them for use with all of the above applications. The Macintosh is an advanced 32-bit design with many advantages over more primitive 16-bit personal computers.

PROGRAM MAINTENANCE

There are now more than 60 minicomputers in the program; many of these systems have been in use for years. The programs used on these systems were written by a number of people, many of whom are no longer in the IRP. Design of these programs is such that changes are usually required as the experimental protocol develops, so program maintenance is a continual and time-consuming function of the Branch. Structured programming techniques and standardization of equipment have enabled the Branch to provide these services without an increase in personnel.

MICROPROCESSORS

The Branch also maintains a microprocessor development system for the software and hardware development of microprocessor-based instrumentation at both the chip and single board computer level. The system currently supports three common microprocessors; one 16-bit processor, and two 8-bit processors. These microprocessors and their associated peripheral chips are now available in CMOS low power versions. This development allows the design of both smaller, more reliable bench instruments and more intelligent portable instrumentation.

The performance of the development system has been enhanced by the addition of a 5M byte Winchester disc. This hard disc provides faster access time as compared to the floppy discs which it replaced and its greater storage capacity allows direct access to all system and commonly-used application software.

IMAGE PROCESSING SYSTEM

The Branch maintains a general purpose image processing system consisting of an Optronics rotating drum film scanner, a DeAnza image array processor, and a PDP-11/60 computer. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using images generated by CAT or ECAT scanners. A camera station is available to generate color hardcopy using Polaroid SX-70 or 35 mm film.

Interactive, menu driven, software packages have been developed to provide an extensive and expandable repertoire of basic image processing functions. Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. The two primary applications of the system are the densitometric analysis of autoradiographs of brain or tissue sections and the analysis of two-dimensional electrophoresis gels.

The Branch is developing a prototype image processing system which will be capable of using these software packages, but will be smaller and less expensive to purchase and operate than the PDP-11/60 based system. It will be based on a PDP-11/23 computer and use a TV camera for digitizing instead of the rotating drum film scanner.

VAX COMPUTER SYSTEM

The Branch manages a multi-user VAX-11/750 computer system that is available for use by all investigators in the IRP. The VAX is located in Bldg. 36, in space furnished by the Laboratory of Cerebral Metabolism. Potential users in Bldg. 36 may request installation of hard wired cable connections, or the VAX may also be used on a dial-up basis.

A device independent graphics package has been developed on the VAX that permits plots to be generated on numerous display terminals and hardcopy devices. A terminal emulation program is available which permits small PDP-11 laboratory computers to function as graphics terminals when using the VAX. This program also supports file transfers in both directions.

The SPICE2 circuit analysis program has been installed on the VAX and is being used by several investigators for modeling neuronal circuits. Programs have been written to generate graphical displays and hardcopy plots of the output of the SPICE2 program.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care is staffed by a veterinarian, laboratory animal technicians, and animal caretakers who provide daily care and maintenance of laboratory research animals. Services provided by the Section include:

(1) Management of the Central Primate Facility. The Central Primate Facility has a capacity for approximately forty primates. At the present time, three investigators have research projects in progress. Support is provided through

assistance in preparing surgical models, insertion of catheters, and maintaining animals on special diets and feeding routines. The Section has also developed the capability for bacteriological culturing and identification, pH and blood gases, and non-invasive blood pressure monitoring.

(2) Tuberculosis Testing Program. All primates, including those located at St. Elizabeth's Hospital, are tuberculosis tested every three months.

(3) Treatment of sick and post-operative animals.

(4) Procurement of all animals for the Division of Intramural Research, NIMH.

(5) Providing day-to-day care and maintenance of research animals. Care and maintenance of research animals are provided seven days a week in all areas. The supervisor of the Section provides day-to-day contact with personnel in all work areas and coordinates weekend and holiday schedules. Night coverage of the primate nursery continues to work very smoothly with two part-time persons covering on alternating nights. Also, these two persons administer night medications to animals on treatment.

(6) An orientation program is being developed for new investigators and technicians by NIH. Although only in the planning stage, it should be functional in the near future.

(7) Animal facilities. Upgrading and additions to the animal holding facilities continue. Renovations and additions to Building 9 will be completed by 1 October 1984. Only a limited number of animals are being housed in the third and fourth floors of the ACRF due to inadequate ventilation. Plans have already been formulated to correct the problem with work scheduled to start the latter part of this year.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Branch's workload among the various laboratories and branches. We have listed only the major users.

LABORATORY OR BRANCH	HOURS	PERCENT
Neurophysiology, NIMH	2551	11.00
Clinical Psychobiology, NIMH	2444	10.55
Psychology and Psychopathology, NIMH	1910	8.24
Neurophysiology, NINCDS	1874	8.09
Neuropsychology, NIMH	1668	7.20
Cerebral Metabolism, NIMH	1340	5.78
Biophysics, NINCDS	1299	5.61
Neural Control, NINCDS	1086	4.69
Clinical Science, NIMH	1063	4.58
Adult Psychiatry, NIMH	970	4.18
Neuropathology and Neuroanatomical Sciences, NINCDS	840	3.62
Biological Psychiatry, NINCDS	660	2.84
Preclinical Pharmacology, NIMH	633	2.73
General and Comparative Biochemistry, NIMH	497	2.14
Neurochemistry, NINCDS	426	1.84
Molecular Genetics, NINCDS	373	1.62
Neurobiology, NIMH	366	1.57
Surgical Neurology, NINCDS	345	1.49
Clinical Neuroscience, NIMH	272	1.17
Molecular Biology, NINCDS	233	1.01
Clinical Neuropharmacology, NIMH	180	.77
Experimental Therapeutics, NINCDS	155	.67
Neurochemistry, NIMH	116	.50

*NIMH	(Total)	14,777	63.30
*NINCDS	(Total)	6,704	28.61
*NICHD	(Total)**	1,875	8.09
		23,356	100.00

*These figures represent our total effort; they include time for labs not listed individually.

**NICHD loans the Branch one position and is thus entitled to 1700 hours of service.

ANNUAL REPORT OF THE INTRAMURAL RESEARCH PROGRAM AT SAINT ELIZABETHS HOSPITAL
National Institute of Mental Health
October 1, 1983 to September 30, 1984

Richard Jed Wyatt, M.D., Associate Director

Once again, it is my pleasure to try to bring you up to date on life in the Intramural Research Program at Saint Elizabeths Hospital. Part of the rewards of my position is to work towards the future, while at the same time, seeing earlier efforts come to fruition. One of the projects that is moving towards completion successfully is the creation of sections within the Program. During the past year we have designated sections to be headed by Drs. William Freed, Daniel Weinberger and Joel Kleinman. These groups have already become extremely active. They will greatly strengthen the Program and we look forward to completing this reorganization process in the next few months.

The cerebral blood flow machine has been operative for a year now and approximately 650 tests have been run. This addition to our laboratory promises to produce valuable new data. Also new, the rennovation of the Animal House is now complete and we have moved back in. This facility has greatly enhanced our basic research activities. We are currently gearing up to shift all wards to one side of the building and to begin remodeling laboratories. Rearranging the building so that laboratories are on one side and the wards are on the other, along with other fire safety code improvements we have made, will insure a superior safety level for our patients and staffs.

During the past year, Dr. John Morihisa, of the Adult Psychiatry Branch, won the A.E. Bennett Award. Dr. Morihisa has followed in the footsteps of Dr. William Freed, who won the previous year's award. This year also, I was awarded the Daniel Efron Award by the American College of Neuropsychopharmacology. Dr. Erminio Costa, Chief of the Laboratory of Preclinical Pharmacology, has continued as Professorial Lecturer in Pharmacology at the George Washington University Medical School and delivered the Williams Lecture at Emory College and the Plenary Lecture to the American Society of Pharmacology and Experimental Therapeutics. Also, Dr. Peter Bridge was recently honored with the J.D. Lane Award for outstanding medical research, presented by the Public Health Service.

There has been considerable transition in our Administrative Office over the past year. Last summer, Mr. Carl Pergler left to take a position with the Navy. He had been our Administrative Officer for six years and had, during his tenure with us, excercised many techniques that had enhanced the efficiency of the Program. On his leaving, Ms. Catherine Bowie took over his position on a termporary basis and performed in exemplary fashion. Recently she, too, has left the Program for a new position and we wish both Ms. Bowie and Mr. Pergler much success in their new jobs.

Citing some scientific highlights, cerebral flow measurements have demonstrated that there is a decrease in frontal blood flow on activation in schizophrenic patients. Also, this year produced the successful publication of Dr. Luis de Medinaceli's technique to repair peripheral nerves in the rat, restoring considerable function. Dr. de Medinaceli has received widespread publicity for these activities. Dr. Costa's laboratory has continued to make inroads into the mysteries of many opiodes as well as providing new insights into the mechanisms of molecular biology. During the past year we have had 24 guest workers from 6 countries. We have invited 50 speakers and published over 150 papers.

Finally before I close, let me mention a matter of continued and growing concern to those of us in the Program at Saint Elizabeths Hospital. We are responsible to three administrative groups: Saint Elizabeths Hospital, where we are housed and to which our patients are admitted; the NIMH through the Intramural Research Program; and the National Institutes of Health. That subjects us to the problems of all three Institutions. For example, during the last year there was a substantial reduction-in-force at Saint Elizabeths Hospital, a percentage of which had to be taken out of our nursing, housekeeping and dietary staff. At the same time there was a small loss of positions in the Intramural Research Program, which also produced a loss in our Program. Thus, during the year we lost positions both on the Saint Elizabeths and the Intramural Research Program sides. This, combined with increasing rules and regulations in almost every direction we step, places a heavy burden on the Program. As I write this there is a freeze on all hiring within the NIMH, making it difficult to replace those who leave. Being responsible to three different organizations, combined with the increasing amount of paper-work, and the loss of support staff makes maintaining the Program at peak performance trying. Our staff recognizes that this is a difficult situation. Fortunately, we have dedicated scientists and staffs, committed to producing research of the hightest calibre. As I look to the coming year, my hope is that circumstances will coalesce in such a way as to further enhance, rather than detract from, this committment.

ANNUAL REPORT OF THE ADULT PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1983 to September 30, 1984

Richard Jed Wyatt, M.D., Chief

Research in the Adult Psychiatry Branch covers a wide range of biopsychiatric and biomedical subspecialities such as neurochemistry, neuropsychiatry, neuropsychology, psychopharmacology, neuroanatomy and neurophysiology. The talents of our researchers in these diverse but interrelated disciplines are most heavily focused on illumination of the etiologies, mechanisms and treatment of the schizophrenias and the major psychiatric disorders of the aged.

Scientifically, the 1983-1984 reporting period has been another exciting and productive year for our Program. On the heels of Dr. William Freed's win last year, Dr. John Morihisa won the A.E. Bennett Award this year for his BEAM research. This award winning research was the first combined brain imaging investigation of schizophrenia to employ both BEAM and computerized axial tomography (CT) technology. This work was performed in the newly operational Neurophysiology Laboratory that Dr. Morihisa has established. The laboratory consists of equipment to record EEG's and evoked potentials, as well as high-speed data processing and a computerized topographic mapping system that displays electrophysiologic data in condensed and summarized form. We look forward to much work emanating from this new facility.

Also within our Schizophrenia Program, another piece of equipment that has generated much excitment and interesting data is our new regional cerebral blood flow machine. For example, Drs. Berman, Weinberger, Morihisa and Zec have been studying unmedicated schizophrenic patients performing cognitive tasks. Preliminary results indicate that approximately 40-50% chronic schizophrenics became hypofrontal when asked to perform the Wisconsin Card Sort Test, while none of the normal controls had this reaction. These findings are being further investigated in larger schizophrenic populations.

In our investigations into the side-effects of neuroleptic drugs, Dr. Dilip Jeste's work over the past year has lent support to the hypothesis of noradrenergic hyperactivity, rather than postsynaptic dopamine receptor supersensitivity, in tardive dyskinesia. In one of Dr. Jeste's studies, CSF samples were collected from 28 psychiatric inpatients in Bombay, India. In the subsequent blind analyses of the samples of eight patients with tardive dyskinesia, five with spontaneous dyskinesia and 15 without dyskinesia, patients with TD had significantly higher norepinephrine concentrations in the CSF compared with the other two groups. Dr. David Shore has been involved, also, in an ongoing collaboration with the United States Secret Service studying White House cases. The goal of this work is to improve prediction of future violent behavior of delusional mental patients.

In our Geriatric Psychiatry Program, Dr. Luis de Medinaceli's work on nerve repair has continued to produce exciting results. Dr. de Medinaceli has found, preliminarily, that autoimmune response is much less severe after lesions are repaired by reconnection. Our brain graft work in Parkinson's disease, also, has expanded. Increasingly sophisticated means of histologic examination have been applied, resulting in a more highly refined understanding of the grafting process in our host animals. Also, we have continued to consider the interrelated issues surrounding this process, especially those issues pretaining to ethical considerations and procedures for applying these transplantation techniques to humans.

Finally, this year marks the conclusion of our Drugs of Abuse Program. While such research continues to be important, both psychiatrically and medically, we feel that our Division's resources are best concentrated in our remaining two programs. The work that concludes our Drug Abuse studies is Drs. Hashtroudi, Bridge and Parker's research on memory and alcohol consumption. The results of this work, demonstrating differential effects of alcohol on memory function, will be expanded, I am certain, in many other laboratories.

This past year's work is the result of the combined efforts of an outstanding and unusually talented group of scientists and staffs. As in each previous year, this year's work has been produced by those who have been with us for many years, some who have just joined us and others who have since left.

This year we say good-bye to Drs. William Lawson, who leaves to take a faculty position at the University of California at Irvine and Steven Potkin, who is also at Irvine. Dr. Esa Korpi has returned to Finland. Cathy Bowie and Kathy Hughes from our Administrative Office and Cindy Owen have left also. We wish all of them much future success. We welcome Drs. Wisniewski, Lohr and Cadet. Finally, I wish to close by recognizing the superb performance of our secretarial and Administrative Office staffs. Both have, and continue to, weather a difficult transitional period with dedication to their responsibilities and coworkers. In sum, the Adult Psychiatry Branch continues to be a pleasant, productive Program of which I am proud to be Chief.

Annual Report
Laboratory of Preclinical Pharmacology
October 1, 1983 thru September 30, 1984
Laboratory Chief: Erminio Costa, M.D.

During FY 1984, we became concerned with the sharp decline in the number of psychiatric drug discoveries we had experienced during the last decade and therefore began to search for the reasons for such a decline. We thought that probably the approaches followed in drug development needed renovation. It has frequently been stated that serendipity played a major role in drug development. But perhaps, serendipity has been overemphasized, because in all drug discoveries there has always been a chemically or biologically oriented effort. Chemically-oriented efforts consist of screening structural modifications of successful drugs for the purpose of improving their potency and specificity. However, seldom have chemically guided efforts led to novelty and often they have been bogged down by tedious screening procedures designed to uncover analogs of existing drugs. Since the search for novelty can be pursued more successfully with biological approaches, we wondered whether one could tailor a biological approach to pursue such a goal. A major focus for psychiatric drug discovery is synaptic transmission, which transacts communication among the various neurons. Until recently, this communication was conceived as a single signal process and drug discovery strategies depended on agonists, antagonists, or metabolic modifiers of the single chemical signal (transmitter) that was believed to transact synaptic communication. However, it became clear that the use of stable analogues of these transmitters lacked functional specificity, and prolonged use of transmitter antagonists triggered unwanted side effects. More recently, it became known that synaptic transmission is a multiple signal process, with mediatory and modulatory chemical signals interacting with each other to confer functional specificity to given synapses. It appears that in order to preserve functional specificity of responses one should focus on modulatory signals and develop drugs that are analogues, antagonists or specific metabolic modifiers of modulatory signals. The question then is: how can we identify those modulatory signals operative in affecting the function of the synapses whose failure to function properly causes the symptoms of psychoses? An answer to the question came from studies with anxiolytic benzodiazepines and two antidepressants, imipramine and mianserin. In these studies, using as a probe the high affinity binding of these drugs to specific neuronal recognition sites, we could uncover important basic mechanisms where these drugs act. Because of the therapeutic efficacy of these compounds, their high affinity site of action is bound to be per se relevant for the discovery of brain mechanisms operative in those symptoms of psychiatric diseases that these drugs relieve.

Following this strategy in benzodiazepine studies, Drs. A. Guidotti, M.G. Corda, (Guest Researcher), M. Ferrari (Guest Researcher) P. Ferrero (Guest Researcher), and Mr. D. Konkel discovered a 10K dalton polypeptide (diazepam binding inhibitor, DBI) which probably is the precursor of a modulator neuropeptide of about 18 amino acid residues which acts on those GABAergic mechanisms ($GABA_A$) operative in regulating the levels of anticipatory anxiety. This regulation, presumably, has a survival value. In collaboration with Dr. J. Bormann (Max Planck Inst., Gottingen, Germany) we have discovered that this peptide acts on the GABA regulated Cl^- channels by reducing the rate of gate openings elicited by GABA; in the absence of GABA, DBI is devoid of action. In other words, its action is opposite that of the benzodiazepines. Moreover, in collaboration with Drs. Herbert and Civelli (Dept. of Chemistry, University of Oregon at Eugene, Oregon) we are working on a DNA that transcribes for mRNA (poly A^+), which has encoded the structure of DBI.

Experiments carried out by Dr. M.L. Barbaccia (Guest Researcher) by applying a similar strategy to the study of high affinity binding of radioactive imipramine, have led to the discovery that this molecule acts on the recognition site for an endogenous endocoid which modulates serotonin (5HT) uptake. Thus, modulation of this reuptake and alteration of the modulator of this uptake may be operative in causing some of the symptoms of depression. The endogenous agonist for this site appears to be a derivative of isoxazole whose structure we are currently investigating with mass fragmentography in a collaborative study with Dr. F. Karoum (Adult Psychiatry Branch, NIMH).

The studies of Drs. B. Roth (Guest Researcher), T. Nakaki (Visiting Fellow), and D.M. Chuang with radioactive mianserin and ketanserin led to the discovery of multiple molecular forms of a 6K dalton polypeptide which appears to be a precursor of the endogenous agonist of the so-called $5HT_2$ recognition site. This site appears to be coupled to serotonergic transmission. By using cloned cell cultures containing $5HT_1$ and $5HT_2$ recognition sites, we are attempting to elucidate the functional consequences of the receptor coupling on serotonergic synaptic function.

Another problem of therapeutic relevance in the relief of pain by the opioids is the phenomenon of narcotic tolerance and dependence. Using a strategy similar to that used for the benzodiazepines, Drs. H.-Y.T. Yang, J. Tang (Visiting Fellow), J. Chou (Visiting Fellow), and M. Iadarola (PRAT Fellow) are revealing that narcotic tolerance is not a receptor modification phenomenon but instead is related to accessory mechanisms such as a release of endogenous peptides endowed with naloxone-like activity. Perhaps, this release is triggered by the persistent occupation of the opioid recognition sites following repeated administrations of high doses of synthetic oplate receptor ligands. Such a release appears to be more important in triggering tolerance than receptor modifications caused by the continuous occupancy of opiate receptor sites by an agonist.

Section on Neuroendocrinology (A. Guidotti, M.D., Section Chief)

1. Pharmacological and physiological implications of neurotransmitter coexistence in adrenal chromaffin cells.

The tenet that chromaffin cells of adrenal medulla specialize exclusively in producing, storing and secreting catecholamines was widely accepted during the last fifty years. It was also believed that these processes were modulated exclusively by nicotinic receptors located in the chromaffin cell membranes. However, since recent evidence has challenged this simplistic view, the necessity for a thorough up-dating of this classic concept has been stimulated by numerous findings, including the documentation of the presence of opioid peptides, substance P, somatostatin, VIP and histamine in adrenal medulla and the demonstration that opiate and substance P receptors are located on chromaffin cell membranes. Moreover, functional studies have led to the belief that intrinsic mechanisms are operative in adrenal medulla physiology; in fact, persistent activation of opiate or substance P receptors down-regulates the nicotine-induced release of catecholamines. This evidence indicates that adrenal chromaffin cells might be an ideal model to study the processing, release and receptor function of opioid peptides derived from proenkephalin-A coexisting with catecholamines as putative neuromediators or modulators.

During the last year we have used this model to study how cAMP modulates gene expression of tyrosine hydroxylase through nuclear protein phosphorylation involving translocation of activated cytosolic protein kinase. Concomitantly protein kinase translocation also activates the expression of gene coding for proenkephalin A, a precursor of enkephalin-like peptides. Dr. Y. Kageyama (Guest Researcher) showed that indeed 8-Br-cAMP and forskolin, an agent that stimulates adenylate cyclase, raises the cyclic AMP content in adrenal chromaffin cells and elicits a significant increase of enkephalin-like peptides. This increase involves an activation of DNA transcription of proenkephalin A, which presumably is triggered by the nuclear translocation of activated catalytic subunits of cytosolic cAMP-dependent protein kinase. This evidence, together with the demonstration by Dr. T.T. Quach (Visiting Associate) that 8-Br-cAMP increases the content of mRNA that codes for proenkephalin A, makes the adrenal medulla model a very attractive tool to study gene regulation at the rate limiting step for transmitter and cotransmitter biosynthesis.

Recently, Drs. Y. Kataoka (Visiting Fellow), Y. Gutman (Visiting Scientist), I. Hanbauer (Pharmacologist, NHLB, NIH), P. Panula (Visiting Fellow), B. Wise (Staff Fellow) and J.Y. Wu (Dept. Physiology, Pennsylvania State University, Hershey, Pa) have observed that the adrenal chromaffin cells contain µM concentrations of GABA. The adrenal chromaffin cells also contain glutamic acid decarboxylase, GABA transaminase and GABA receptors. The GABA receptors located on the membranes of chromaffin cells have similar properties as the brain $GABA_A$ postsynaptic receptors. They bind with high affinity to ^{3}H-muscimol, ^{3}H-flunitrazepam and ^{35}S-t-butylbicyclophosphorothionate, and these specific recognition sites interact with each other with modalities similar to those described for brain GABA/benzodiazepine recognition sites.

Bicuculline facilitates the nicotine-induced release of catecholamines from chromaffin cells but fails to change the KCl-induced release of catecholamines. These studies confirmed an interaction between GABA and nicotine receptors by showing that nicotinic receptor stimulation releases the GABA stored in chromaffin cells. This release, in turn, may reduce catecholamine secretion elicited by nicotine. We have not yet established whether GABA plays a role in the regulation of enkephalin release. However, in dogs, a significant physiological role of the GABA receptors in the control of catecholamine release from adrenal chromaffin cells is supported by a bicuculline-induced facilitation of the catecholamine release elicited by electrical stimulation of splanchnic nerve. Hence, if intrinsic GABAergic mechanisms modulate chromaffin cell response to ACh released from splanchnic nerve, one might suggest that polar GABAergic drugs may be developed to decrease adrenal chromaffin cell responses to stress. The role of enkephalin-like peptides released in stress is not clear, but an action on natural killer cells appears to be operative. We are now studying this problem as a possible link between mood alteration and facilitation in the onset of neoplastic processes.

2. Role of GABA/benzodiazepine receptor complex in the neurobiology of conflict regulation.

Our present understanding of the role of the benzodiazepine recognition sites and their endocoids in regulating anxiety has been complicated by the emergence of beta-carboline-3-carboxylate esters, a new class of benzodiazepine recognition site ligands. Dr. M.G. Corda has shown that these ligands facilitate the onset of conflict in rats. Others have shown that they cause panic anxiety in man. Using a simple modification of the Vogel test, Dr. M. G. Corda has shown that it is possible to determine whether a benzodiazepine receptor ligand is a proconflict or anticonflict agent and, thereby, infer whether a given endocoid or drug is a potential anxiolytic or anxiogenic agent in man. Dr. M.G. Corda's experiments are in line with a number of reports from several laboratories confirming the theory first advanced ten years ago by our laboratory that the anxiolytic and anxiogenic action of benzodiazepine recognition site ligands is mediated by a facilitation or attenuation of GABA receptor function, respectively.

Binding experiments performed in our laboratory, and in other laboratories, with crude synaptic membrane preparation of rat brain support the view that benzodiazepines increase, and beta-carbolines decrease, the Bmax of GABA recognition sites. To verify whether this in vitro modification of the Bmax of GABA recognition sites by benzodiazepines or beta-carbolines was operative in vivo, Dr. P. Ferrero (Guest Researcher) has studied the ex vivo binding of ^{3}H-muscimol to brain membranes prepared from various brain structures of mice receiving diazepam (1 mg/kg i.p.). She has shown a marked increase of ^{3}H-muscimol being elicited by diazepam. This experimental model may give us the opportunity to verify whether the pharmacological potency of various anxiolytic drugs relates to their ability to increase the Bmax of GABA recognition sites. Dr. Ferrero has reported that ^{3}H-muscimol, injected intravenously into the mouse, binds to high affinity postsynaptic GABA recognition sites located in various brain structures. In addition, by using this approach, one can establish basic models to study the GABA receptor function in man with positron emission computed tomography scanning.

We continued our studies on the characterization of the brain endocoid acting on the benzodiazepine recognition site by using a behavioral test, which is a modification of the Vogel test where thirst drives appetitive behavior and an electric current applied to the water spout is used as punishment. In collaboration with Drs. C. Forchetti (Guest Researcher), M. G. Corda, Mr. D. Konkel and Dr. Bennett (Merck Sharp and Dome, Phila., Pa.) we purified to homogeneity and partially sequenced a 105 amino acid residue neuropeptide which displaces ^{3}H-diazepam and ^{3}H-beta-carbolines from high affinity recognition sites located in neuronal membranes. The behavior elicited by intraventricularly injected DBI has a proconflict profile similar to that of FG 7142, a derivative of beta-carboline-3-carboxylate, which is anxiogenic in man. DBI even reverses the anticonflict action of diazepam when administered intracerebroventricularly to the rat.

Drs. Ferrero and Corda have shown that DBI can be cleaved by cyanogen bromide and trypsin in small fragments that are active in inducing proconflict action in animals, thus suggesting that DBI might be a polyprotein functioning as a precursor of an endogenous putative neuromodulator of benzodiazepine/GABA receptor system. Current data support the view that the endocoid for the benzodiazepine recognition site may have 18 amino acid residues, but we do not know yet whether DBI contains one or more replicas of this endocoid. An implication of these findings is that the brain has a mechanism capable of producing anxiety by decreasing GABA synaptic function, presumably in selected brain areas. In line with the participation of GABA in the modulation of anxiety states, Drs. M. Ferrari (Guest Researcher) and P.Ferrero have shown that the proconflict action of DBI and FG 7142 is increased several-fold when the efficiency of GABAergic transmission is attenuated by the injection of isoniazid, a drug which reduces GABA availability by inhibiting the activity of glutamic acid decarboxylase. Recently, we have obtained an antiserum toward DBI which allows for a determination of DBI in brain tissue. The antibodies contained in this serum interact with the CNBr fragment of DBI which is biologically active. Though we have not yet identified the various molecular forms of DBI present in brain tissue, we can say that DBI-like immunoreactivity is the highest in cerebellum, the lowest in striatum, and intermediate contents are present in cortex, hippocampus and midbrain. Human brain contains biologically active DBI, which immunologically and by HPLC can be identified with rat DBI. Studies on the action of DBI on GABA-mediated regulation of Cl^- fluxes have been performed by Dr. J. Bormann with cell membrane fragments of primary cultures of hippocampal pyramidal cells. The results of these experiments suggest that DBI down regulates the rate of Cl^- gate openings caused by GABA; however, in absence of GABA, DBI is inert. In collaboration with Drs. Herbert and Civelli, we are cloning cDNA for DBI. We intend to determine the amino acid sequence of DBI and prepare a cDNA probe to estimate DBI synthesis rate in vivo.

The molecular mechanisms that couple GABA with benzodiazepine recognition sites and the mechanism that allosterically modulates Cl^- ionophore opening when GABA sites are occupied in addition to the sites which recognize picrotoxin, participate in the supramolecular organization of GABA receptors. To unravel the functional aspects of the GABA receptor organization we began to dissect single components of this receptor system and study them in simplified models. Dr. A. Novelli (Guest Researcher) has initiated the purification and isolation of benzodiazepine recognition sites and has purified this protein by 1000 to 2000-fold. Dr. F. Vaccarino (Guest Researcher) has been successful in characterizing a basic polypeptide with 130 amino acid residues present in synaptic membranes in close association with GABA recognition sites. This polypeptide, termed GABA-modulin, is a noncompetitive allosteric inhibitor of GABA binding. GABA-modulin is similar to the small basic protein present in myelin (SMBP); however, it can be differentiated from SMBP because it is present in the synaptosomal fraction of rat brain, has a higher molecular weight than SMBP, and has a different amino acid composition. Since polyclonal antibodies raised against GABA-modulin cross-react with SMBP, Drs. F. Vaccarino and M.J. Dobersen (Biochemist, NINCDS, NIH) have prepared monoclonal antibodies against GABA-modulin in order to study the physiological role and localization of this protein in brain. NZB/N mice have been immunized and their lymphocytes fused with P3x63 myeloma cells. One of the cloned hybrids was selected because it secretes antibodies specific for GABA-modulin which fail to cross-react with either SMBP or myelin basic protein in concentrations 100-fold higher. These antibodies have been used by Drs. F. Vaccarino and V. Gallo (Istituto Superiore Research, Rome, Italy) to detect GABA-modulin in primary cultures of granule cells from rat cerebellum which is particularly rich in GABA receptors. These cells in primary culture are not myelinated while the cultures contain less than 5% of astrocyte and oligodendrocyte cells. These primary cultures represent an excellent model to study the GABA-benzodiazepine/Cl^- receptor/ionophore complex and its regulation because they contain postsynaptic recognition sites for GABA, benzodiazepines and ^{3}H-t-butylbicyclophosphorothionate. These binding sites can be detected either in intact cell or in disrupted cells. Granule cells contain a protein that when purified by a standard procedure, has an identical molecular weight and amino acid composition as purified GABA-modulin from whole brain synaptosomes. This protein also reacts with our specific monoclonal antibody and its concentration in granule cell primary cultures is 0.52 μg/mg protein.

The presence of GABA-modulin in a homogeneous population of granule cells which do not use GABA as a neurotransmitter, but receive strong GABA input from Golgi cells, is highly indicative of the postsynaptic localization of GABA-modulin and suggests that this protein functions as a coupler in the supramolecular organization of GABA receptors. We are confident that the availability of a monoclonal antibody for GABA-modulin will pave the way to new studies on the functional role of this protein.

Section on Molecular Neurobiology (E. Costa,M.D., Acting Section Chief)

1. Immunochemistry of adrenergic receptors

Dr. D.M. Chuang continued his efforts to produce monoclonal antibodies to frog red cell membrane components, in an attempt to use them as tools in order to reach a better understanding of the regulation of beta-adrenergic receptor function. He has raised monoclonal antibodies using as an antigen crude preparation of beta-adrenergic receptor recognition sites solubilized from purified membranes of frog erythrocytes. Screening for antibody production is made by using immunoprecipitation of the solubilized beta-adrenergic receptors labeled with a high affinity receptor ligand, ^{125}I-hydroxylbenzylbenzylpindolol (IHYP). One of the monoclonal antibodies produced has been extensively characterized. When the antigen receptor preparation is fractionated by Sephadex G-150 column chromatography, two binding peaks with different molecular weights are resolved, but only the activity peak with the higher molecular weight can be immunoprecipitated. Using purified plasma membranes, this antibody affects the isoproterenol-sensitive adenylate cyclase biphasically; at low concentrations, the antibody stimulates the isoproterenol-sensitive cyclase activity, whereas at higher concentrations, the activity is inhibited. Immunoblotting of an isoelectrofocussing gel of the antigen indicates that the immunoglobulin is bound to a single spot with a pI=6.2. Moreover, immunoprecipitation of the total erythrocyte protein labeled with ^{125}I reveals that the antibody interacts with a major protein with roughly 43K dalton; these parameters indicate that the antibody is not directed toward the frog beta-adrenergic receptor recognition site but, perhaps, against a membrane protein component which interacts with the beta-receptor recognition site. Hence, it might be a useful tool to study the interaction between the recognition site of beta-adrenergic receptors and other proteins that are part of the supramolecular structure of beta-adrenergic receptors.

2. The insulin-like brain proteins.

Insulin and/or insulin-like polypeptides have been detected in the brain and in peripheral tissues of rats using radioimmunoassays. Previously we had presented evidence that insulin may be a neuromodulator in the dopamine system of rat olfactory bulb. However, it is still unclear whether insulin like material is synthesized in the brain. Drs. R. Lee and D.M. Chuang have carried out hybridization experiments on poly A$^+$ RNA from brain and other tissue with a cloned DNA fragment of proinsulin gene (supplied by Dr. Villa-Komaroff, University of Massachusetts) to assess the presence of insulin mRNA species in rat tissue. There are one to five mRNA bands (with sizes of 560, 630, 860, 1200, and 1500 nucleotides) hybridizing to the probe in various rat tissues. The two bands with the smallest molecular weights are found in the pancreas. In the brain, a third band with 1500 nucleotides is also detected. The specific hybridization is strong in olfactory bulb and tubercles, and in the hypothalamus, hippocampus, brain stem, frontal cortex, striatum and cerebellum but is weak in occipital cortex and spinal cord. mRNA from human brain shows two hybridization bands with sizes of 860 and 2000 nucleotides and mRNA from brains of pig and cow shows three hybridization bands with sizes of 1370, 2660, 3770 nucleotides. These results indicate that transcription of genes for insulin and/or insulin-like polypeptides may occur in extrapancreatic tissues and the transcriptional products are highly heterogeneous in size, suggesting that posttranscriptional processing on RNA may operate differently in different tissues and among various species. Further studies are in progress on the biosynthesis of insulin and/or insulin-like polypeptides in extrapancreatic tissues.

3. Endacoids to $5HT_2$ receptors

Two regulatory sites have been detected in serotonergic synapses of brain, the $5HT_1$ recognition site is linked to adenylate cyclase or to ionophores, whereas the so-called $5HT_2$ receptor is not innervated by serotonergic terminals though it binds 5HT with low affinity. Moreover, in addition to $5HT_2$ receptors 5HT synapses contain a binding site for mianserin, an atypical antidepressant. To obtain further information on the interaction between $5HT_1$, $5HT_2$ and mianserin binding sites, Dr. T. Nakaki (Visiting Fellow) has initiated a study using NCB-20 cells (a mouse neuroblastoma x Chinese hamster brain hybrid cell line) which has been reported to possess $5HT_1$ recognition sites linked to adenylate cyclase. He found that $5HT_1$ sensitive adenylate cyclase can be effectively blocked by ketanserin, a selective $5HT_2$ receptor blocker. Utilizing crude membranes (48,000xg pellets) prepared from cultured NCB-20 cells, he detected ^3H-ketanserin and ^3H-mianserin binding sites with kinetic constants comparable to those reported in brain. NCB-20 cells also take up 5HT in a saturable manner and this uptake process is partially sodium dependent and inhibited by imipramine, fluoxetine and desipramine.

The sites labeled by ^3H-mianserin and ^3H-ketanserin could be merely acceptor sites devoid of physiological significance. To evaluate whether or not these sites have a physiological role, Dr. B. Roth (Guest Researcher) initiated a search for an endocoid for the $5HT_2$ recognition sites. Bovine forebrain was homogenized in 1 M acetic acid, centrifuged to remove insoluble proteins, and applied to a CM Sephadex column. Fractions were assayed for inhibition of ^3H-ketanserin binding and those fractions exhibiting the highest inhibitory activity (ketanserin equivalent/mg protein) were combined. The active fractions were successively purified with a Sep-Pak column and eluted with 70% acetonitrile, and then applied to a Biogel P-10 column. One major active fraction (MW approximately 6K dalton) was collected and then applied to a C-18 reverse phase HPLC column followed by ion exchange HPLC. Preliminary studies suggest that the HPLC eluate yields biological activity and chromatographs as one band in PAGE. However, in these PAGE studies, due to the low molecular weight of the compound, it is difficult to exclude that low molecular weight fragments elute with the 6K dalton biologically active peptide. With the most active fractions, as little as 1-3 μg of the partially purified peptide, inhibits at least 50% of the ^{3}H-ketanserin specific binding. In preliminary studies specificity for the $5HT_2$ recognition sites labeled with either ^{3}H-mianserin or ^{3}H-ketanserin was noted. There was little specific inhibition of beta-adrenergic, imipramine or 5HT binding to rat cortical membranes. We have also assessed the protease sensitivity of the partially purified peptide. Preincubation of the peptide fraction with either pronase (a mixture of exo- and endopeptidase) or trypsin partially reversed the inhibitory activity.

4. The endocoid regulating 5HT uptake

Drs. M.L. Barbaccia, O. Gandolfi (Guest Researcher), L. Ravizza (Guest Researcher) and Dr. O. Pozzi (Guest Researcher) continued their investigation on the role of serotonergic innervation in the down regulation of imipramine, ketanserin and mianserin recognition sites elicited by various antidepressants known to act either on the ^{3}H-imipramine recognition sites or on the ^{3}H-mianserin recognition sites. All the antidepressants, regardless of whether or not they act on the imipramine recognition site, down regulate the beta-adrenergic receptor recognition sites. If an antidepressant down regulates the imipramine recognition sites, its down regulation of the beta-adrenergic receptor function is dependent on the presence of serotonergic axons, but this dependency does not exist if the compound acts on the ^{3}H-mianserin recognition sites. Thus, all the antidepressants given for an extended time period appear to decrease the function of 5HT synapses, some by acting presynaptically (^{3}H-imipramine recognition sites), others by acting postsynaptically (^{3}H-mianserin recognition sites). Through this attenuation of 5HT function, they alter signal amplification at the beta-adrenergic synapses by a transsynaptic mechanism which originates in the 5HT terminals, and its location and biochemical specificity remains to be clarified. In order to reach such a clarification, Dr. M.L. Barbaccia investigated whether high affinity imipramine binding sites regulate 5HT upake in brain slices. Hippocampal slices prepared from rats receiving daily treatments of imipramine for three weeks have a reduced number of imipramine binding sites. These sites appear to regulate 5HT uptake because when ^{3}H-imipramine binding is reduced, 5HT uptake is increased. In hippocampal and striatal minces from the same brain, uptake of dopamine or norepinephrine is not modified. These results suggest that the imipramine binding site is functionally coupled in an inhibitory manner to 5HT uptake. Dr. M.L. Barbaccia has successfully extracted from whole brain an endocoid that selectively inhibits the binding of ^{3}H-imipramine and neuronal 5HT uptake. This putative endogenous ligand (endocoid) for the imipramine binding site is extractable from brain with either acetic acid, formic acid or methanol and it appears to be stable in a temperature of 90°C for 15-20 minutes. It is soluble in methanol and ethanol, but not in propanol and acetonitrile. The biological activity of the endocoid is resistant to pronase and trypsin digestion suggesting that it is not a protein. However, it can be completely separated from endogenous 5HT by HPLC on a reverse phase column. Among the various indole related compounds tested for their ability to inhibit ^{3}H-imipramine binding and ^{3}H-5HT uptake, only the tetrahydro-beta-carboline derivatives (6-hydroxy and 6-methoxy tryptoline) were found to be active with IC_{50} values of 0.8 μM and 0.2 μM, respectively. Although these compounds appear to be selective inhibitors of the ^{3}H-imipramine binding and ^{3}H-5HT uptake, they cannot be suggested as possible candidates for the role of endogenous ligands(s) for the ^{3}H-imipramine binding site because their elution profile from the reverse phase HPLC column is strikingly different from that of the putative endocoid being extracted from the rat brain.

5. DNA transcription and neuropeptide turnover

In order to understand which neurotrophic factor acts in the development of a given type of neuron, Drs. J. Schwartz, and T.T. Quach (Visiting Associate) are attempting to clone the gene of that factor(s) whose synthesis is stimulated following a brain mechanical lesion. This factor is characterized by its ability to support neuronal survival in dissociated cultures of 12 day chick embryo sensory or sympathetic ganglia. The mRNA prepared from lesioned brain is converted to DNA (cDNA) with reverse transcriptase and hybridized with mRNA (poly A^+) from control brain to remove all the sequences expressed in control brain. The mRNA (poly A^+) pool which is not hybridized includes all the messages specific to the lesioned brain and following in vitro translation this mRNA is screened for the presence of neurotrophic factors using the chick embryo dissociated neuronal cultures of sensory ganglia. The types of messages present in the lesioned brain cDNA library will be of general interest in order to understand the molecular events involved in brain injury. New immunocytochemistry studies in lesioned and pathological states will open up.

Drs. I. Mocchetti (Guest Researcher) and J. Schwartz have proposed a novel molecular biological approach that allows for the estimation of the changes in the in vivo turnover rate of neuropeptides in various brain areas. At this time, studies have been carried out with two families of opioid peptides, the enkephalins and proopiomelanocortin. The method is based on the simultaneous measurement of mRNA for the polyprotein functioning as the neuropeptide precursor, and the precursor of the biologically active neuropeptide. The mRNA contents were measured using specific cDNA probes for proenkephalin and proopiomelanocortin (donated by Dr. E. Herbert, University of Oregon, Eugene, Ore) on a (poly A^+) mRNA preparation of the brain area. The peptide content was measured before and after trypsinization of the acetic acid extract of the brain area purified by column chromatography to separate the high from the low molecular weight form of immunoreactive peptides. While the immunoreactivity of the low molecular weight material fails to increase after trypsinization, the high molecular weight peptide immunoreactivity increases following trypsinization. This is due to the unmasking of biologically active neuropeptides binding with high affinity to the antibody. At times, depending on the structure of the processing signal, the immunoreactivity increases further if trypsinization is followed by an additional step: digestion with carboxypeptidase B. The increase in the immunoreactivity of the high molecular weight fraction following trypsin and carboxypeptidase digestion expresses the precursor content, while the immunoreactivity of the low molecular weight which does not change following trypsinization expresses the content of biologically active neuropeptides. In areas innervated by dopaminergic neurons daily injections of haloperidol repeated for two to three weeks increase the mRNA content for proenkephalin, the enkephalin precursor, and the content of biologically active enkephalin. These results taken together indicate an increase in the enkephalin turnover because the number of specific mRNAs and their expressions are increased. Haloperidol also selectively increases the mRNA content coding for POMC in the intermediate lobe of pituitary. Also in this case, to assess whether this indicates an increase in the beta-endorphin turnover rate, Drs. I. Mocchetti and J. Schwartz are measuring the POMC and beta-endorphin contents. When hypothalamic serotonergic function is impaired, the enkephalin content of this area is increased selectively. Since the hypothalamic content for the mRNA for proenkephalin is not increased, Drs. I. Mocchetti and J. Schwartz suggested that this increase in enkephalin reflects a reduction in peptide utilization rates. To confirm this inference they are now studying whether posttranscriptional processing is increased.

Drs. J. Schwartz, O. Giorgi (Guest Researcher), W. Fratta (Visiting Scientist) and I. Mocchetti have reported that in rat made tolerant to morphine, or when withdrawal is precipitated by naloxone, there is an increase in the hypothalamic mRNA (poly A^+) for proenkephalin, without an increase in the mRNA for POMC. We are now assessing posttranscriptional processing in order to be able to infer whether there is a change in opioid peptide biosynthesis during tolerance or withdrawal.

Catecholamines and enkephalin coexist in adrenal chromaffin cells; hence, as previously stated in this report, one can study the regulation of the biosynthesis of coexisting neuropeptide and monoamine transmitters. Dr. J. Naranjo (Guest Researcher) is measuring mRNA for tyrosine hydroxylase (TH) and proenkephalin at various times after treating primary chromaffin cell cultures with 8-Br-cyclic AMP. Both of the mRNAs were accumulated following second messenger application. Also dexamethasone causes a dose dependent increase of the mRNAs for TH and proenkephalin. However, when the cells are treated with reserpine, a divergence in the response of the two mRNAs is seen. The catecholamine content and the proenkephalin mRNA decrease, whereas the enkephalin content and the mRNA for TH increase. These results suggest that reserpine decreases enkephalin utilization by chromaffin cells while it blocks the storage of catecholamines. As a result, the catecholamine content is reduced and mRNA for TH is increased to accelerate synthesis. In contrast, the mRNA for proenkephalin is decreased to reduce synthesis and balance the decreased utilization of this neuropeptide. Our current working hypothesis is that mRNA synthesis of coexisting transmitters is modulated by a dual control: transsynaptic stimuli and utilization rates. Probably, when the latter is decreased mRNA transcription is blocked. Only when utilization is increased can transsynaptic stimuli via second messenger activation accelerate transcription. We are carrying on experiments to evaluate the validity of this working hypothesis.

6. Neurochemical correlates of behavioral responses of septal-hippocampal cholinergic neurons.

In rats, septal cholinergic neurons are regulated by a number of afferents, axons originating from extrinsic neurons and interneurons which are contiguous to the cell bodies of septal hippocampal cholinergic neurons. Considering that memory and behavior depend on hippocampal cholinergic tone, Dr. W. Blaker has probed the role of such regulation by studying behavioral and biochemical effects of the GABA agonists, muscimol, beta-endorphin and substance P, three known modulators stored in the cholinergic afferents. To avoid reflex actions, he has injected these modulators intraseptally and in conjunction with behavioral observations has studied acetylcholine turnover rate (TR_{ACh}) in hippocampus. Muscimol decreases the TR_{ACh} only in the ventral hippocampus, whereas substance P decreases it only in the dorsal hippocampus. Dr. W. Blaker has hypothesized that lowering the cholinergic input to the ventral hippocampus increases extinction responding, whereas a decrease in the input to the dorsal hippocampus is without this effect. Hence, the cholinergic projections to the two hippocampal areas are modulated by different transmitter systems and have different physiological functions.

Dr. W. Blaker has also characterized GABAergic control of the cholinergic pathway projecting from substantia inominata to the frontal cortex of the rat. It is known that activation of GABA receptors in the substantia inominata by microinjection of muscimol decreases the TR_{ACh} in the cortex. To test the hypothesis that such control may be tonic, in separate experiments, Dr. W. Blaker injected bicuculline (a GABA antagonist) into the substantia inominata and destroyed the major GABAergic input to the region by lesioning the nucleus accumbens with kainic acid. Neither treatments altered the TR_{ACh} in the cortex indicating that the GABAergic control of this cholinergic projection is similar to that in the septal hippocampal pathway which is not tonic.

7. Protein phosphorylation as a transsynaptic regulatory mechanism.

Dr. B. Wise has been working on protein phosphorylation as a transsynaptic regulatory mechanism in the function of the GABA/benzodiazepine receptor complex. GABA-modulin (GM) as discussed earlier in this report appears to be operative in the modulation of the number of GABA recognition sites. GM has been found to be an excellent substrate for various protein kinases. The cAMP-dependent enzyme phosphorylates GM at 4 distinct sites, whereas a Ca^{2+}/calmodulin-dependent protein kinase phosphorylates GM at 2 sites, one of which is different from the cAMP-dependent sites as revealed by reverse phase HPLC of tryptic digests of GM. Phosphorylation of GM by the cAMP-dependent enzyme, but not by the Ca^{2+}/calmodulin dependent process, results in a loss of GM inhibitory activity towards GABA binding. GM is also a substrate for the recently described Ca^{2+}/phospholipid-dependent protein kinase which phosphorylates GM at 3 sites, two of which are different from the cAMP and Ca^{2+}/calmodulin-dependent sites. The effect of the Ca^{2+}/phospholipid-dependent phosphorylation on GM activity is unknown at present. Such intramolecular modifications of GM by these enzymes may provide a novel mechanism for the fine tuning of the GABA receptor complex. To extend these findings to an intact cell preparation, the bovine adrenal chromaffin cell was chosen, since studies by Dr. Y. Kataoka demonstrated the presence of an intrinsic GABAergic system in primary cultures of these cells. Dr. Wise has shown that there is a GM-like protein present in membranes prepared from adrenal medulla and chromaffin cells. This GM-like protein is present in a phosphorylated state in chromaffin cells incubated with ^{32}P to label ATP pools. In addition, Dr. Wise has demonstrated the presence of protein kinases activated by cAMP and Ca^{2+}/phospholipid in cytosol and membrane fractions of adrenal medulla and chromaffin cells. The existence of endogenous protein substrates, some of which have the same molecular weight as GM, for protein kinases of various natures in both cytosol and particulate fractions suggests that the adrenal chromaffin cell is a suitable model to study the transsynaptic regulation of protein phosphorylation as it relates to the GABA receptor complex.

Section on Neuropeptides: (H.-Y.T. Yang, Ph.D., Section Chief)

1. Naloxone-like endocoid in CNS: Drs. M. Iadarola, J. Tang (Visiting Fellow), and H.-Y.T. Yang attempted to evaluate the biological role of enkephalinergic pathways in spinal cord by measuring the distribution and release of met^5-enkephalin-arg^6-gly^7-leu^8 (MERGL). Similar to other carboxy extended forms of enkephalins derived from preproenkephalin A, MERGL is highly concentrated in the dorsal gray of spinal cord. However, Met^5-enkephalin and Met^5-enkephalin-arg^6-phe^7 immunoreactivity are represented mostly by the small molecular weight forms of the penta- and octa-peptide, while MERGL immunoreactivity consists of

roughly 50% of the authentic octapeptide and 50% of the high molecular weight form of N-terminal extended MERGL. Interestingly, both molecular forms of MERGL-like immunoreactivity can be released by either: 1) intrathecal application of substance P or 2) bilateral electrical stimulation of the sciatic nerve. Probably, substance P is released from axon terminals of 'C" fiber primary afferents included in the sciatic, and this peptide, in turn, releases MERGL stored in interneurons containing substance P receptors. This is consistent with the view that small molecular weight MERGL, as well as its high molecular weight form, are released by nociceptive stimuli reaching the spinal cord via the 'C" fibers. The functional implication of the release of high molecular weight material is not clear at the present time. It is possible that the high molecular weight form may act to prolong the action of MERGL by inhibiting the MERGL degrading enzyme competitively or by serving as a MERGL precursor. Drs. H.-Y.T. Yang and J. Tang have previously demonstrated that the antinociception elicited by met^5-enkephalin-arg^6-phe^7 (MERF) or morphine can be reduced by either FMRF-NH_2 when injected intraventricularly or intrathecally, or by CCK-8-SO_4 injected intrathecally. Proglumide, a weak CCK receptor blocker, antagonizes the antiopiate activity of CCK-8-SO_4 as expected, but surprisingly also blocks the antiopiate activity of FMRF-NH_2. A naturally occurring FMRF-NH_2 immunoreactive material was found but it is different from authentic FMRF-NH_2. In order to assess the role of endogenous polypeptides endowed with FMRF-NH_2-like immunoreactivity in opiate action, antinociception thresholds were measured in rats receiving parenteral proglumide, a weak CCK receptor antagonist, or intraventricular IgG isolated from FMRF-NH_2 antiserum. Both proglumide and the IgG potentiated morphine antinociception in rats and inhibited the development of acute morphine tolerance induced by seven to eight injections of morphine repeated at 2 hr intervals. Furthermore, a higher dose of IgG from FMRF-NH_2 injected intrathecally induced a moderately long lasting and naloxone reversible antinociception. A similar but weaker effect was also observed when the IgG was injected intraventricularly. IgG prepared from control serum was devoid of the above mentioned activities in antinociception. These results seem to suggest a functional role for the endogenous FMRF-NH_2 immunoreactivity in opiate nociception; however, they fail to exclude the participation of CCK-8-SO_4 or other endogenous neuropeptides endowed with opiate antagonist activity in the modulation of nociception thresholds by endogenous opiate peptides. ACTH and MSH neuropeptides, and some chemical congeners, may act as naturally occurring opiate antagonists. Interactions between ACTH-MSH-like peptides and opiates were studied by using the electrically stimulated myenteric plexus longitudinal muscle of the guinea pig. Dr. W. Fratta found that gamma$_1$MSH inhibited the electrically induced contractions of guinea pig ileum myenteric plexus longitudinal muscle and this effect was reversed by naloxone. Furthermore, gamma$_1$MSH potentiated the effect of beta-endorphin and leu^5-enkephalin in this intestinal muscle preparation. These results suggest that gamma$_1$MSH is endowed with opiate activity that can be antagonized by ACTH (1-24). Alpha-MSH and ACTH (4-10) are also active but are less potent in antagonizing the opiate activity of gamma$_1$MSH. In crude synaptic membranes prepared from rat brain, gamma$_1$MSH specifically displaced ^{3}H-dihydromorphine, ^{3}H-ethylketocyclazocine and ^{3}H-DAla^2-D-Leu^5-enkephalin binding with an IC$_{50}$ of around 10^{-7}M further documenting the affinity for opiate receptors of gamma$_1$MSH. These results taken together raise the possibility that gamma$_1$MSH may coexist with endorphin in certain axons to facilitate the action of endorphin on specific receptors; in contrast, ACTH and MSH may modulate the responsiveness of these receptors by down regulating with the recognition sites for beta-endorphin or gamma$_1$MSH.

The location of FMRF-NH_2 immunoreactivity containing neurons and the effect of IgG prepared from FMRF-NH_2 antiserum on opiate analgesia imply a functional role for the neuropeptide endowed with FMRF-NH_2 immunoreactivity in the modulation of nociception threshold. The exact molecular structure of the neuropeptide endowed with FMRF-NH_2-like immunoreactivity stored in mammalian neurons still needs elucidation. Dr. H.-Y.T. Yang characterized the FMRF-NH_2 immunoreactivity in various rat brain regions. In rat, the brain content of neuropeptides with FMRF-NH_2 immunoreactivity (expressed as fmol FMRF-NH_2 equivalent/mg protein) is: cerebellum, 5.4±0.7; medulla oblongata, 16±1.3; striatum, 5.4±0.6; midbrain, 13±1.4; hippocampus, 3.5±0.5; hypothalamus, 35±3.6; cortex, 4.7±0.7; pituitary, 102±11; spinal cord, 40±2.7). HPLC analysis of various molecular forms of FMRF-NH_2 immunoreactivity revealed three different forms of immunoreactive peptides with retention times of 28 min (A), 32 min (B) and 40 min (C). All three forms are distinct from FMRF-NH_2, gamma_1MSH and NPY. Interestingly, the amount of each form varies greatly in different brain regions. For example, content of C form is high in spinal cord and medulla oblongata but rather low in hypothalamus and pituitary. Whether all three forms have a similar biological activity is an interesting question that remains to be answered.

2. Enzymatic processing of opioid peptides

Dr. I. Lindberg continued to study the trypsin-like enzyme which she had shown to generate enkephalin from high molecular weight enkephalin containing peptides. This enzyme was further purified and characterized by using several endogenous peptides or proteins as substrates. An enzyme preparation from bovine medullary chromaffin granules purified 1000-fold cleaves the purified 5.3K dalton N-terminal extended met-enk-arg-gly-leu purified from proenkephalin-A polypeptides and yields met-enk-arg-gly-leu. Purified human kininogen, a precursor of kallidin and bradykinin, was also used as substrate for the enzyme; no cleavage leading to bradykinin or kallidin was observed. The results clearly differentiate the enkephalin generating enzyme purified from adrenal from the purified kallidin. The short basic enkephalin-like neuropeptides, such as met^5-enkephalin-arg^6-phe^7, met^5-enkephalin-arg^6-gly^7-leu^8 and dynorphin (1-8) were not hydrolyzed by this enkephalin generating enzyme. These results taken together suggest a physiological role for this trypsin like enzyme isolated from chromaffin granules in the processing of proenkephalin-A to enkephalins.

Previously, we demonstrated that met^5-enkephalin-arg^6-phe^7 (YGGFMRF) is metabolized by dipeptidyl carboxypeptidase and aminopeptidase. With the cooperation of Dr. S. Blumberg from the Weizmann Institute of Science, Rehovat, Israel, Drs. J. Chou and H.-Y.T. Yang continued to search for effective enzyme inhibitors to block the met^5-enkephalin-arg^6-phe^7 inactivation. Three mercaptoacylated dipeptides, HS-CH_2-CO-Leu-Phe, HS-CH_2-CO-Phe-Leu and HS-CH_2-CO-Leu-D-Phe were provided by Dr. S. Blumberg as potential enzyme inhibitors. HS-CH_2-CO-Leu-Phe effectively inhibited the inactivation of YGGFMRF by dipeptidyl carboxypeptidase in vitro. To estimate the in vivo effect of this inhibitor, the recovery of YGGFMRF released by substance P into spinal cord subarachnoidal space was measured. HS-CH_2-CO-Leu-Phe, when added into the perfusion medium, was found to increase the release of YGGFMRF. Interestingly, the recovery of YGGFMRF released into spinal cord was increased by subcutaneous injection of CH_3-CO-S-CH_2-CO-Leu-Phe-OCH_3. Whether this inhibitor is capable of reaching the CNS after subcutaneous injection still remains to be established.

Several endogenous opioid peptides, derived from proenkephalin are metabolized mainly by aminopeptidase and dipeptidyl carboxypeptidase. The dipeptidyl carboxypeptidase can be effectively inhibited by captopril; however, this drug is not capable of penetrating the blood brain barrier. Dr. B. Melstrom (Visiting Fellow) has examined many dipeptidyl carboxypeptidase inhibitors 3(supplied by Revlon and Hoechst AG) in inhibiting the inactivation of met^5-enkephalin-arg^6-phe^7 in vitro. She has found some potent inhibitors such as Rev 5511 (IC_{50}=1 μM); Rev 5229-3 (IC_{50}=2.5 μM) from Revlon. The inhibitor, Hoe 498-disaure from Hoechst AG is very potent in inhibiting the inactivation of met^5-enkephalin-arg^6-phe^7 by dipeptidyl carboxypeptidase in vitro. This drug, Hoe 498-disaure and its ester, Hoe 498 will be examined for their activity in inhibiting met^5-enkephalin-arg^6-phe^7 metabolism in vivo. Whether these inhibitors can penetrate the blood brain barrier will also be studied.

Section on Biochemical Pharmacology, (N. H. Neff, Ph.D., Section Chief)

1. Transducer systems for adenosine (A_1) and GABA$_B$ recognition sites

Dr. W. Wojcik (Guest Researcher) has determined that adenosine A_1 and GABA$_B$ are negatively coupled to cyclase. These receptor systems have separate recognition sites and separate coupling mechanisms, but share common cyclase catalytic units acting as transducers. Hence, stimulation of adenylate cyclase with supramaximal concentrations of agonists for either recognition site induces the same activation as adenylate cyclase when the two agonists are together or when added individually. These receptors appear to be located on nerve terminals that release excitatory transmitters, such as glutamate, aspartate and acetylcholine. GABA$_B$ and adenosine A_1 agonists generally hyperpolarize neurons and prevent neurotransmitter release.

2. Neurochemical measurements of neuronal depolarization

Dr. D. Cavalla (Visiting Fellow) has observed that undifferentiated neuroblastoma x glioma cells (NG-108-15) accumulate fluorescent cyanide dye DiO-C_5-(3) to a degree dependent on their membrane potential. With a dye concentration of 25 mM, the fluorescence increases with hyperpolarizing agents and decreases with depolarizing agents, a new equilibrium being established within 10 min. For example, 1 μM valinomycin, a potassium ionophore, increases fluorescence whereas potassium reverses this effect; that is, depolarizes in a concentration-dependent manner. Gramicidin, a channel-forming quasi-ionophore depolarizing agent, decreases fluorescence. As would be expected, membranes from cells lysed by sonication do not show these changes. In more recent studies, Dr. Cavalla has shown that opiate peptides hyperpolarize neuronal cells and this response can be blocked with opiate antagonists. Indeed, the membrane potential-fluorescent dye technique may be the simpliest method to evaluate the consequences of neurotransmitters on the membrane potential of neuronal cells.

3. Photoaffinity labelling of transmitter recognition sites

Dr. D. Cavalla has continued his studies of photoaffinity labeling of transmitter recognition sites. Tritium labeled 2-azidoadenosine was synthesized as a potential candidate to label either adenosine A_1 or A_2 receptors. When irradiated, the compound irreversibly attaches to membranes prepared from brain tissue. Unfortunately, binding was not to the adenosine receptor, it appeared to be linked to the adenosine transport site. Thus, this new compound may prove to be a useful tool for characterizing the adenosine transport protein. The known photochemistry of compounds similar to muscimol, a potent $GABA_A$ agonist, was applied to obtain the photolabelling of $GABA_A$ receptor. Polyacrylamide gel electrophoresis revealed a molecular heterogeneity among GABA binding proteins, confirming evidence from binding studies suggesting two different affinity sites for GABA and its analogs in rat brain crude synaptic membranes.

4. Peripheral dopamine receptors

There is substantial evidence for dopamine receptors in the cardiovascular system. These receptors are located either postsynaptically, Type DA-1, primarily on renal and mysenteric vascular beds and their activation leads to myorelaxation or prejunctionally, type DA-2, on noradrenergic axonal varicosities and their activation leads to reduced release of norepinephrine. Dr. M. Hadjiconstantinou (Guest Researcher) has provided experimental evidence that dopamine receptors are probably innervated by dopaminergic neurons. She has shown that dopamine and norepinephrine can be depleted differentially from the cardiovascular system of the rat with the neurotoxin, 6-hydroxydopamine, in combination with the amine uptake blocking drug desipramine or with the selective noradrenergic neurotoxin, N-(2-chloroethyl)N-ethyl-2-bromobenzylamine (DSP-4). Moreover, she has demonstrated that the distribution of norepinephrine, dopamine and DOPAC in human blood vessels is consistent with the notion that the two catecholamines are stored in separate sites.

5. Modulation of retinal catecholamine receptors

Environmental light activates dopamine containing neurons in rat retina and as a consequence dopamine turnover increases. The state of dopamine metabolism is directly related to the retinal content of DOPAC. Dr. M. Hadjiconstantinou found that alpha-2 adrenoceptors are present in the retina and their activation diminishes the retinal content of DOPAC in rats placed in the light, but not of rats placed in the dark. When $alpha_2$ antagonists are administered they increase retinal dopamine metabolism of rats in the light as well as of rats in the dark. These observations are consistent with the notion that an endogenous agonist fully occupies the $alpha_2$ receptor in the dark and only partially occupies the receptors in the light. The most likely endogenous agonist for these receptors is epinephrine. Indeed, Dr. M. Hadjiconstantinou together with Dr. A. Mariani (Staff Fellow, NEI) localized phenylethanolamine-N-methyltransferase, the enzyme for the synthesis of epinephrine, by an indirect immunofluorescent staining method to a subpopulation of amacrine cells in the rat retina. The cells are located primarily in the inner nuclear layer and send a single process to the inner plexiform layer. These epinephrine-containing amacrine cells are morphologically distinct from the dopamine-containing amacrine cells previously described by formaldehyde fluorescence.

6. Mice genetically prone to convulsions as a pharmacological model for epilepsy

The compound, N-methyl-1-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a by-product of the synthesis of meperidine, induces neurological symptoms in man and primates similar to Parkinson's disease, including the loss of dopamine from the nigrostriatal system. Indeed the compound may provide an animal model for studying Parkinson's disease. Drs. M. Hadjiconstantinou, H. Laird and H.

Anthcpoulou (Guest Researcher) have found that MPTP as well as some analogues induce a long term decrease of dopamine and norepinephrine in regions of mouse brain. Moreover, there is a concomitant elevation of acetylcholine in these regions of brain. Of significant importance is the finding that dopamine in the spinal cord and retina are also reduced.

The genetically epilepsy prone rat (GEPR) is a valuable model that allows for the exploration of neurological mechanisms which case the genetically determined seizure-prone state of epilepsy. There is a growing body of data showing abnormalities in the norepinephrine, serotonin and possibly GABA transmitter systems in these rats. In March, Dr. H. Laird began establishing a colony of these animals for LPP and also began a systematic study of their neurobiochemistry. Preliminary data suggest that acetylcholine is elevated in many regions of brain where the content and turnover of norepinephrine is markedly lower than normal. The establishment of the GEPR colony will provide us with the opportunity to evaluate the molecular bases for a neurological disorders.

Group on HPLC Instrumentation in Neurochemistry (James Meek, Ph.D., Head)

Drs. J. Meek and C. Eva (Visiting Fellow) have been working to improve an HPLC method to measure ACh tissue content that was developed by Drs. P. Potter, J. Meek and N. Neff, during the previous fiscal year. A crucial step of this method was the post column reaction of ACh with enzymes to produce peroxide, which, in turn, was detected electrochemically. It was found that enzymes could be easily fixed by adsorption to an anion exchange cartridge, and that the absorbed enzymes were stable for weeks. This simple sensitive system has aroused considerable interest. Using this method, Dr. C. Eva has been studying the action on ACh turnover rate of neuropeptides coexisting with ACh in the parasympathetic innervation of mouse salivary gland. In these nerves, there appears to be a co-localization of vasoactive intestinal peptide (VIP) and ACh in almost every axon. Dr. C. Eva found that muscarinic agonists and antagonists decrease and increase, respectively, salivary gland ACh turnover, suggesting a negative feedback loop. VIP also decreased ACh turnover. Experiments are in progress to determine whether VIP can act when cholinergic receptor function is blocked by muscarinic antagonists. Furthermore, it will be investigated whether VIP modulates the function of autoreceptors or acts on the feedback modulatory loop.

Dr. J. Wroblewski (Visiting Fellow) has been working on the problem of the synthesis of the neurotransmitter pool of glutamate. This amino acid may be the most common excitatory compound in brain, but no techniques exist to study its regulation by tracing its dynamics. These studies are impaired by our inability to identify the precursor for the neurotransmitter pool. Dr. J. Wroblewski using rat septal nuclei as a model found that a blockade of the enzyme ornithine aminotransferase led to a rapid decline in the concentration of a small amount of glutamate, and that this septal pool of glutamate could be largely eliminated by a lesion of the incoming glutamatergic pathway. He has extended this work by showing that while radioactive ornithine and alpha-ketoglutamate can both be incorporated into glutamate, the kinetics of incorporation are quite different and suggest that ornithine may be a precursor of glutamate pool functioning as a neurotransmitter. The experiments will now be extended to include measurements of releasable glutamate in vivo using in push-pull experiments the newly developed dialysis probes, and the exquisite sensitivity of HPLC.

Dr. F. Nicoletti (Guest Researcher) has been studying hormonal modulation of GABAergic function using the hypothalamic pituitary system as a model. The pituitary possesses GABA receptors but it cannot synthesize GABA. Probably GABA is released from hypothalamic neurons into the portal circulation, it reaches the pituitary receptors where it acts to modulate the release of prolactin and other hormones. He has found that repeated estrogen treatment causes a massive (6-10 fold) increase in pituitary GABA content. He is currently investigating whether this increase is the result of hyperprolactinemia and thereby represents part of a feedback circuit.

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